NOAA Technical Memorandum NMFS

SEPTEMBER 2009

ESTIMATION OF POPULATION SIZE WITH MOLECULAR GENETIC DATA

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NOAA-TM-NMFS-SWFSC-448

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
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Abstract

Population size is a central parameter in ecology, evolutionary biology and conservation. Until the advent of molecular genetic methods, population size was measured through observation and/or capture of individuals. Now, molecular data are frequently used to estimate size. There are several definitions of population size, which differ mainly with respect to the temporal, spatial and genealogical scale of reference. Those interested in the number of individuals usually study the census size, whereas those primarily interested in the genetic consequences of population size generally study the effective size. The various definitions of effective size are largely defined in reference to a Wright/Fisher or "ideal" population. We provide an overview of the different definitions of population size and of the methods to estimate them using molecular genetic data, with an emphasis on recently developed methods for estimating effective size. These methods can use genetic samples from single or multiple points in time. They can also estimate effective size in a defined interval or an indeterminate one that ranges from several generations to the entire coalescent history of the genetic sample. We also discuss methods for evaluating changes in size, both long-term changes and recent reductions or bottlenecks. We argue that the scale of reference is crucial in the choice and interpretation of a size estimation method. Specifically, we note that when estimating and reporting a population size investigators should 1) specify precisely the time period and spatial area over which the size is believed to prevail, 2) remember that different genetically-based methods for estimating effective size do apply to specific time periods and time scales, 3) note that none of the three main effective sizes—inbreeding, variance, or eigenvalue—are intrinsically associated with a particular time scale.

Introduction

Population size is one of the most fundamental parameters in ecology, conservation and evolutionary biology. It is a primary determinant of population dynamics and of the relative importance of deterministic and stochastic evolutionary forces on ecological interactions, population trajectory and extinction risk. In biological management and conservation, it is frequently the basis for harvest regulation and conservation action. In population genetics and evolutionary biology, it determines the rates of inbreeding, genetic drift, and loss of neutral genetic variation.

There are several ways to define population size, and the particular definition applied is generally reflective of the interests of the investigators and the general themes in their biological discipline. In ecology and conservation, biologists are often interested in the absolute number of individuals in a spatially-defined area or phylogenetic lineage at a specific point in time. Such traditional abundance, or *census*, population sizes are important for understanding density-dependent relationships and evaluating extinction risk due to demographic factors (Lande 1988). Evolutionary biologists and population geneticists are typically more interested in the evolutionary consequences of population size and thus have focused on concepts of population size that are related to the number of individuals passing genes from one generation to the next, or the genetically *effective* size. In addition, many investigators, particularly in conservation, are interested primarily in detecting and estimating changes in population size, or *relative* population size, over time or space (e.g. founder events).

The temporal, spatial and genealogical scale of study must be carefully defined in order to understand the many methods for the estimation of population size and properly interpret their results. The temporal scale of interest ranges from a specific point in time, usually the present, for the census and some effective size estimators, to the "evolutionary" history of the population for many of the effective size estimators. The spatial scale of investigation varies from a "closed" or local population, to the entire species or global population. The genealogical scale of interest varies from a specific set of pedigreed individuals to the entire set of coalescent events in the ancestry of a genetic sample.

This large range of scales of interest has led to a proliferation in the literature of estimators and definitions of population size that may measure different quantities over different scales but carry the same name. Accordingly, direct comparison of estimates derived using different methods can lead to disparities and disagreement**.** This is due not only to the fact that census and effective sizes of the same population may be markedly different (Frankham 1995), but also because different estimators of effective size can apply to different scales and may, therefore, estimate different underlying quantities. These disparities can be resolved by careful consideration of what is being measured and of the specific scale of relevance, which, we argue, must always be made explicit when interpreting and reporting population size estimates.

Traditionally, biologists estimated population size using demographic and life-history data collected through capture and/or field observation. In the last several decades, the development of molecular genetic methods has led to a variety of estimation techniques that draw inference about population size from a sample of variable molecular markers

such as DNA sequences, allozymes or microsatellites. In the past decade, improvements in molecular biology techniques and population genetic analysis have led to numerous methodological advances in the estimation of population size from such data. The advent of large-scale microsatellite and single nucleotide polymorphism (SNP) genotyping in non-model organisms has greatly expanded the abundance of large genetic data sets for use in such estimation. Advances in population genetic inference have included the use of the coalescent and of Bayesian methods in analyzing such data. Refinements in the design of Markov chain Monte Carlo (MCMC) and importance sampling algorithms, as well as the increased power of desktop computers, have made computationally intensive likelihood-based and Bayesian inference procedures commonplace (Luikart & England 1999).

Here we provide an overview of the methodology currently in use for estimating population size, in its various formulations, using molecular genetic data. We aim to provide a guide for population biologists and molecular ecologists interested in using genetic data to estimate population size and interpret the estimates properly. Our summary should also be sufficiently comprehensive that it can be used as a point of reference for population geneticists who are interested in a summary of the major concepts and methods. We focus on methods resulting from recent advances in molecular methods, statistical procedures, and computing power. We describe the differences between various definitions of population size and discuss the importance of the Wright-Fisher model in understanding population size definitions and estimators. We also discuss the issues of scale that are crucial in understanding and interpreting results from such

estimates, and provide guidelines for when different methods might be most appropriate or informative.

We begin with a brief discussion of recently developed methods for estimating census size using genetic data. We then provide an overview of the concept of effective size, reviewing definitions and then describing the various estimators. It is not our goal to provide an exhaustive review of all published definitions and estimators of effective size and their properties. Nor do we summarize the abundant theoretical work in predicting effective size or evaluate published estimates from empirical data. Instead, we focus on methods developed in the last several years, with an emphasis on likelihood-based methods, as earlier methods have been previously reviewed (Schwartz *et al*. 1998; 1999). Finally, we discuss methods for inferring changes in population size and providing information about relative population size.

Census size

Often, investigators are most interested in estimating the number of individuals in a population, or its census size, N^C. If there is a well-defined boundary, which may be geographic, genetic or ecological, this number is generally interpreted as the absolute abundance of the population. In the absence of a clear boundary, the size estimate is actually the population density for a defined spatial area. The traditional method of estimating census size of natural populations is through direct observation of individuals. This can include so-called exhaustive methods in which every individual in a population is observed, catalogued, and counted, or statistical methods such as mark/recapture or line-transect techniques. These methods, and their assumptions, are reviewed extensively

elsewhere (Pollock *et al*. 1990). All of these require direct handling (or observation), making them difficult to implement for many species. For such species, the use of genetic "tags" may provide a feasible alternative.

Genetic data can provide a DNA fingerprint that is essentially an individual-specific, inborn "tag" (Paetkau & Strobeck 1994). Such genetic tags can serve as marks in traditional mark/recapture techniques to estimate N_c (Palsbøll 1999; Palsbøll et al. 1997). In such methods, individual genotype data are collected from two sets of samples from the same location that are serially collected within the same generation. Genotype ("tag") matches are treated as recaptures and used to estimate the absolute number of individuals by making assumptions about equal probability of capturing any individual in both "trapping" events and the absence of migration during the sampling interval, as in traditional mark/recapture analyses. Genotype information can be collected from hair (Garza & Woodruff 1992; Morin et al. 1994), feathers (Taberlet & Bouvet 1991), epithelial cells in feces (Hoss et al. 1992) and other naturally-shed tissues, which can be collected from wild populations non-lethally or even non-invasively without direct handling of the organism. This has allowed the estimation of size for animal populations that are not amenable to trapping or that exhibit behavioral responses to the "mark" phase of mark/recapture, thereby violating key assumptions of such methods.

Another important use of molecular genetic data in estimating N_c is in the elucidation of population structure and the definition of population boundaries. Many taxa contain barriers to gene flow that are not readily apparent, or closely related groups whose individuals are difficult to identify morphologically (e.g. cryptic species). Failure to identify such subdivision within a sample can lead to substantial error in population size

estimates. Specific (i.e. diagnostic) molecular markers have been developed for the identification of many groups with components that are difficult to partition otherwise (Garza & Woodruff 1994; Taggart & Ferguson 1984). In cases where there is no *a priori* knowledge of subdivision, analytical methods which exploit the multilocus genotypes of individuals can be used to determine whether the sample collected for size estimation contains structure (Pritchard et al. 2000; Dawson & Belkhir 2001; Corander et al. 2004; Huelsenbeck and Andolfatto 2007). Recent reviews have addressed these methods and the challenges associated with their use in identifying population boundaries (Pearse & Crandall 2004; Waples & Gaggiotti 2006). When there is reason to believe the sample is not from a single randomly-mating population and there is a reasonable hypothesis for where barriers might exist, such as between different habitat patches, summary statistic (e.g. F_{st}) methods might be sufficient to define such boundaries.

The presence of migrants from other populations poses a special problem. Migrants violate the assumptions of most N_c estimation methods and blur the spatial boundary of populations. Methods which exploit genetic data and likelihood models for the assignment of individuals to population of origin and for the detection of migrants may allow such individuals to be properly considered when estimating census size (Rannala & Mountain 1997; Pritchard et al. 2000; Anderson & Thompson 2002; Wilson & Rannala 2003; Paetkau et al. 2004).

Effective size

Population geneticists have long recognized that the census population size is not the only parameter determining the dynamics of genetic variation in time and space. The

transmission of genes from one generation to the next is a process influenced by a variety of factors, both demographic and otherwise. Explicitly modeling all factors governing the fate of genes in populations is a difficult, if not impossible, task. Moreover, any model that explicitly incorporated all the characteristics of one population might not be applicable to a second population with different demographic and biological traits. For these reasons, population genetic theory has been developed largely in reference to "ideal" population models, with the Wright-Fisher model (Fisher 1922) primary among them.

A Wright-Fisher population is one of constant size in which each individual has an equal probability of producing offspring and has an equal chance of mating with any individual in the population, including itself. Generations are discrete, and all members of the population reach maturity in one generation and die after the opportunity to reproduce once. In other words, the genes of generation t+1 are copies of genes randomly sampled with replacement from generation t. There is only a single variable parameter in this model: population size. The Wright-Fisher model has been central to population genetic theory, because its simplicity allows the mathematical analysis of genetic transmission between generations in finite populations, and it has served as the starting point for investigations of the effects of most population-genetic forces. The model itself, or some elaboration of it, has been used to explore the rate of loss of alleles (Wright 1931), probability of fixation of an advantageous allele (Kimura 1962; Ohta 1972), ability of a population to purge itself of deleterious mutations (Kimura et al. 1963; Gabriel et al. 1993; Glémin 2003), the effects of gene flow, migration, and subdivision (Kimura & Maruyama 1971; Wright 1943) and many other phenomena. Additionally, the details of

the coalescent process governing gene trees in finite, neutrally-evolving populations were first developed by tracing ancestry in the Wright-Fisher model backwards in time (Kingman 1982).

Many biologists and ecologists, however, are interested in real populations where the simplifying assumptions of the Wright-Fisher model are violated. For example, natural populations frequently have overlapping generations, fluctuating population size, over-dispersed variance of individual reproductive success, two sexes, and assortative mating. Although such populations diverge substantially from a Wright-Fisher population, the model still provides a useful approximation for many purposes. In such cases, it is necessary to determine what size of Wright-Fisher population provides the best approximation. This size is the effective size, or N_e, of the population (Wright 1931). It is thus clear why effective size is a crucial characteristic of a population: it provides a link that allows the use of population genetic theory to make statements about the probable fate of genes or genetic variation in real populations.

The effective size of a natural population is defined to be the size of a Wright-Fisher population that would have the same rate of change of a genetic parameter as the real population under study. Different choices of genetic parameter lead to different definitions of effective size. The three most common definitions of effective size are: inbreeding (Wright 1931), variance (Crow 1954) and eigenvalue (Ewens 1979) effective sizes. A population with inbreeding effective size N_e experiences the same rate of increase of the average inbreeding coefficient as a Wright-Fisher population of size N_e. Likewise, in a population of variance effective size N_e, allele frequency variance increases over time at the same rate as in a Wright-Fisher population of size N_e. Finally, a

population of eigenvalue effective size N_e is one whose reproduction is governed by a transition probability matrix for which the largest eigenvalue less than one is equal to the leading non-unit eigenvalue in the matrix describing a Wright-Fisher population of size N_e. In less mathematical terms, two populations with the same eigenvalue effective size will experience the same rate of loss and fixation of alleles over long time periods. The concept of eigenvalue effective size is difficult to apply to natural populations.

In addition, extinction effective size (Neigel 1996), and mutation effective size (Whitlock & Barton 1997) have been described. These are specializations of the three common definitions of effective size for specific scenarios. More recently, several authors have proposed that the effective size can be defined in terms of the rate at which coalescent events occur in the population, leading to the "coalescent effective size" (Durrett 2002, Nordborg & Krone 2002, Sjödin et al. 2005). This concept has been extended by Wakeley and Sargsyan (2009), who argue that coalescent effective size should depend not only on the rate of coalescent events, but also on the re-scaling of time in terms of average generation length. Proponents of the coalescent effective size stress that, "Since the coalescent essentially embodies all of the information that can be found in sampled genetic data, one can argue that if anything deserves the title of 'the effective size' it is the coalescent effective size"(Sjödin et al. 2005, p. 1061). However, given that investigators are frequently interested in genetic processes on time scales other than the very long one which generates patterns of present-day genetic diversity, such a definition seems not sufficiently inclusive.

Much has been done on the theoretical prediction of N_e given various departures from the Wright-Fisher model (e.g. subdivision; Wang & Caballero 1999). This work typically

assumes that characteristics such as variance in family size or relative fecundities of different age classes in the population are known and then provides a formula for N_e given such characteristics (see, for example Crow & Denniston 1988; Hill 1979). Applying such formulae to natural populations can be difficult, however, because the characteristics cited above are typically difficult or impossible to estimate, particularly in highly fecund or mobile species. Consequently, a number of methods for estimating N_e using molecular genetic data have been developed.

In the following, we divide these methods into those using a single sample and those that use two or more temporally-spaced samples. We further divide the methods into those that estimate historical or long-term N_e and those that estimate contemporary N_e (Figure 1). Until the last decade, all methods for estimating N_e used the method of moments or some other summary-statistic-based technique. Recently, there has been a proliferation of likelihood-based and Bayesian techniques, which take advantage of more information in the data. These methods estimate a variety of different quantities over different temporal and genealogical scales. We describe these methods below. Subsequent to the first draft of this report, Wang (2005) published a comprehensive description of the mathematical details of all of these methods, and we refer the reader there when greater detail is necessary or desired.

Single-sample methods

Methods for estimating long-term N_e

Many methods for estimating N_e from a single sample in time have been proposed. Most of the early methods rely on the relationship between N_e and the level of genetic diversity

expected in a population at equilibrium, which is characterized by the population genetic parameter $\Theta=4N_e\mu$ (Ohta & Kimura 1973; Watterson 1975; Nei & Tajima 1981a), where μ is the mutation rate. These methods initially focused on allozyme data or DNA sequences and used a summary statistic calculated from the data (e.g. number of segregating sites), with an assumed mutation rate, to derive an estimate of N_e. Felsenstein (1992a,b) showed that these summary-statistic-based methods are inefficient and proposed the use of a maximum likelihood estimator.

More recently, the use of the coalescent in maximum likelihood and Bayesian estimation of Θ has been extensively developed (Griffiths and Tavaré 1994a; Kuhner et al. 1995; Nielsen 1997; Stephens & Donnelly 2000; Kuhner 2006). These methods typically estimate the N_e prevailing over a long period of time---namely the period until the most recent common ancestor of all gene copies sampled in the population. Many of these methods have been reviewed recently (Crandall et al. 1999; Wang 2005).

Methods for estimating contemporary N_e

Linkage disequilibrium methods

Hill (1981) describes a method for using the observed gametic phase (linkage) disequilibrium between pairs of loci in a single genetic sample to estimate N_e. This method exploits the fact that more disequilibrium is expected to accumulate in populations of smaller size. The estimation technique is based upon the method of moments. By equating the observed, squared correlation coefficient of alleles (r^2) at different loci with its expected value under drift-recombination equilibrium, the estimator

$$\hat{N}_e = 1/[3(r^2 - 1/S)]$$

is obtained for unlinked loci in a population in which mates are non-monogamous. In the above expression, S is the number of diploid individuals in the sample and r^2 is a weighted average, over pairs of diallelic loci, of the estimated square of the correlation coefficient (Hill 1974). The expectation under equilibrium is derived using the limiting distribution of two-locus identity-by-descent measures (Weir & Hill 1980). Accordingly, the method provides an estimate of the inbreeding effective size. However, for unlinked (or only loosely-linked) loci, drift-recombination equilibrium is reached much more quickly than is drift-mutation equilibrium, and Waples (1991) reports that the disequilibrium method estimates the N_e prevailing over only the last several generations (see also Waples 2005). Therefore, the disequilibrium method estimates contemporary inbreeding effective size, instead of long-term inbreeding effective size, as do the estimators based on neutral genetic diversity. This difference provides a good illustration of the fact that inbreeding effective size is not necessarily associated only with long-term or historical effective size.

However, investigation into the behavior of the disequilibrium estimator revealed that it may be biased when sample sizes are small (England et al. 2006). In particular, when the sample size is small, and is also less than the true N_e, the disequilibrium method provides a severely downwardly-biased estimate of N_e. Waples (2006) describes a method for reducing this bias, and Waples and Do (2008) describe a software package, LDNE, which implements this method. However, Russell and Fewster (2009) point out that the method corrects the bias in $1/N_e$ but does not necessarily improve the estimation of N_e. They also conclude, from a series of simulations, that in non-ideal populations, the LD estimator of N_e can perform poorly. It has been suggested that the disequilibrium and

temporal methods (see next section) might be combined to derive a more accurate estimator of N_e (Waples et al. 1993). While we are not currently aware of any such "combination" estimators that exploit temporal samples and disequilibrium, it seems that disequilibrium could be incorporated into approximate Bayesian computation estimation of N_e, described below, without great difficulty.

A related disequilibrium method was proposed by Vitalis and Couvet (2001a,b). This method derives the equilibrium expectations of one- and two-locus identity-by-descent and identity-by-state measures. However, these expectations are calculated for a focal population within an infinite island model with mutation (Vitalis & Couvet 2001c). Their estimator jointly estimates the migration rate between demes and N_e of the focal population. Their simulation results suggest that the method holds promise for data sets with more than 10 loci. However, to our knowledge, the method has only been applied once to a real data set, with mixed results (Wilson et al. 2004). Skalski (2009) describes a related method, which relies on the idea that if juveniles can be sampled after reproduction but before migration, in an infinite island model, it is possible to derive moment-based estimators for N_e and the migration rate separately (rather than only for their product, $N_e m$). He then derives similar estimators for a finite island model under K-allele mutation. Simulation results show that accurate estimates of N_e and m can be obtained when N_e is very small and when one can take large samples (100 individuals) from a large number (20) of demes of identical size. Natural populations with such characteristics and opportunities for sampling are likely to be so uncommon as to preclude the widespread application of these estimators.

Heterozygote excess method

Pudovkin *et al*. (1996) describe a method that exploits the heterozygote excess that arises in small populations of dioecious species to provide an estimate of the effective number of breeders producing a sample of individuals, which must be from the same cohort. While this is not the same as the effective size, it is closely related and can be used to estimate inbreeding effective size. It is based on the principle that in very small populations the limited number of breeding individuals gives rise to random differences in allele frequencies between the two sexes. These differences lead to an excess in the observed number of heterozygotes relative to the expectation under Hardy-Weinberg proportions. The magnitude of this excess is used as a measure of the number of breeders. Obviously, the heterozygote excess estimator applies only to the temporal scale of one generation—that of the parents of the sampled cohort. This method has been shown to provide unbiased estimates of the effective number of breeders only in very small populations (Luikart & Cornuet 1999). However, Balloux (2004) describes how the method may be extended to a highly structured population, in which subpopulations may consist of small numbers of breeders. It is unclear how commonly investigators may encounter species with subpopulations of the required number of breeders (<10) and it is thus unlikely that the heterozygote excess method is of great utility for most species and populations.

In populations small enough that the heterozygote excess method might provide good estimates, a more powerful method of estimating the effective number of breeders would be to assign sampled individuals to full- and half-sibships and directly estimate the number of parents (Thomas and Hill 2002; Blouin 2003; Wang 2004). From such an

estimate, the N_e of the entire population could be derived under assumptions regarding the distribution of offspring (e.g. Hedrick et al. 2000). In fact, a rigorous derivation of such a method was recently presented in Wang (2009). Wang first shows that the inbreeding effective size can be written in terms of the probabilities that a randomly-sampled pair of individuals share a mother or a father and then shows how the fraction of pairs in a sample inferred to be half-siblings and full-siblings yields an estimate of the aforementioned probabilities, providing an estimate of N_e based on sibship analysis. This method is implemented in the program Colony (version 2) which first estimates the maximum likelihood partition of a sample into full-sibships nested within half-sibships and subsequently estimates N_e from the resulting estimated full and half-sibling dyads.

A related approach has been used to estimate the number of breeders in vertebrate populations (Jones & Avise 1997; Pearse et al. 2001). These studies extend the concept of genetic mark/recapture by using parentage analysis and consider the brooding parent as a "trap" in which the "marks" are applied. The "recapture" then occurs either through parentage matches in separate broods, or by comparison of reconstructed parental genotypes with a sample from the parental pool.

Methods using temporally-spaced samples

The temporal method is generically a technique for estimating N_e from the observed change in allele frequencies over time that was first described by Krimbas and Tsakas (1971). The principle behind the temporal method is that the observed degree of allele frequency change provides information on effective size, because random genetic drift occurs more rapidly in a population of small N_e than in a population of large N_e. To use

the temporal method, an initial genetic sample is taken from a population and then another sample is taken after a known number of generations has elapsed. The allele frequencies are then used in a statistical procedure to estimate the population's N_e during the interval between the samples. The main statistical challenge involves separating the "signal" of random genetic drift in the population from the "noise" of random sampling. Thus, the temporal method works best in situations where the amount of genetic drift is large relative to the amount of random sampling error in the genetic samples. More specifically, the temporal method performs best when i) the number of individuals sampled is large, ii) N_e is relatively small, and iii) a moderate number of generations has elapsed in the interval between samples (Waples 1989). Additionally, since each independently-segregating locus provides another replicate of the process of drift in the population, sampling more loci boosts statistical precision. Waples (1989) provides useful rules of thumb as to whether increasing sample sizes or increasing the number of loci will most improve one's estimate.

There has been a remarkable proliferation of statistical techniques for estimating N_e using the temporal method. Until recently, they were all based on the method of moments. In the last few years, however, several likelihood-based approaches have been developed. Most of these methods make a common set of assumptions regarding the population and the sampling under question: i) there is no migration into the population, ii) the loci examined are not influenced by natural selection, iii) mutation is infrequent enough as to be ignored, and iv) the genetic samples are a random sample from an unstratified or unstructured population. In the following, we first review the traditional

moment-based estimators. We then describe the likelihood-based methods that have emerged recently.

Moment-based estimators

The moment-based, temporal-method estimators for N_e follow from the classical theory of the increase over time of the *F*-statistic (Wright 1951) due to genetic drift. In a Wright-Fisher population of size N_e diploid individuals, if the frequency of an allele at time 0 is p_0, it can be shown that the random variable p_t—the allele frequency at time t—has variance such that

$$\frac{\mathrm{Var}(p_t)}{p_0(1-p_0)} = \frac{E(p_t - p_0)^2}{p_0(1-p_0)} \approx \frac{t}{2N_e}$$

when $t/(2N_e)$ is less than about 0.15 (Nei and Tajima 1981b). In other words, the expectation of the squared deviation of p_t from its starting value, p_0, when adjusted to compensate for the effects of its starting frequency [*i.e.*, when divided by $p_0(1-p_0)$], is approximately a linear function of t with slope equal to $1/(2N_e)$. This forms the basis for one formulation of Wright's *F*-statistic:

$$F = \frac{(p_t - p_0)^2}{p_0(1-p_0)}$$

The expectation of *F* is approximately $t/(2N_e)$, so an estimate of *F* from the observed genetic samples may be obtained and then converted into an estimate of N_e.

The estimation of N_e using F-statistics has been well explained and explored by Waples (1989) and has been previously reviewed (Schwartz et al. 1998), so we will not go further into the details of the methods, except to point out some of the problems that they encounter. The primary shortcoming of the F-statistic-based estimators of N_e is their upward bias when low-frequency alleles are encountered in the samples, or when drift between the samples is great enough that the probability that some alleles have drifted to extinction is non-negligible (Waples 1989). This is an especially undesirable feature when using microsatellite markers that may exhibit numerous alleles of low frequency. Jorde and Ryman (2007) propose an alternative allele-weighting scheme for estimating F that gives reduced weight to rare alleles. Using F estimated in this fashion reduces the bias in the corresponding estimator of N_e, but also leads to increased variance of the estimator. In general, however, moment-based estimators do not make full use of the information in the data. For this reason, likelihood-based alternatives for estimating N_e from temporal samples began to be developed in the late 1990s.

Likelihood and Bayesian methods

The first class of likelihood-based methods was based on the hidden Markov-chain model that arises when considering samples taken at discrete intervals from a Wright-Fisher population. Williamson and Slatkin (1999) and Anderson and Thompson (1999) independently developed likelihood-based approaches for estimating N_e using the hidden Markov-chain model. Williamson & Slatkin (1999) proposed directly calculating the likelihood by multiplication of transition probability matrices describing the Wright-Fisher model. They also showed that the likelihood-based estimator is less biased and has

lower variance than the moment-based estimators, that with more than two samples it is possible to estimate an exponential growth rate for a population with N_e increasing over time, and that their method can be extended to markers with dominant expression (e.g. RAPDs and AFLPs). However, their direct calculation is computationally infeasible for loci with more than two alleles, or for very large N_e.

In contrast, Anderson & Thompson (1999) used MCMC for estimating the likelihood curve for N_e. This makes it feasible for multiple alleles, but it is still computationally intensive, and their particular MCMC method was not pursued further. A subsequent paper (Anderson et al. 2000) describes an importance sampling scheme that allows Monte Carlo evaluation of the likelihood for N_e for loci with multiple alleles. This method, implemented in the software package *MCLEEPS*, is still computationally demanding.

Wang (2001) describes another method, implemented in the computer program *MLNE*, for maximum likelihood estimation of N_e from temporally spaced samples. *MLNE* is based on the same hidden Markov-chain model used by Anderson *et al*. (2000), but instead of approximating the true likelihood by a Monte Carlo procedure, it calculates the *pseudolikelihood*. At each locus with k alleles, the pseudolikelihood is the product of k different likelihoods. Each likelihood is computed for a single allele separately with all other alleles lumped into a single category and the locus treated as a diallelic one for which the exact evaluations are computationally feasible (Williamson and Slatkin 1999). In addition to the pseudolikelihood approximation, several other computational efficiencies are incorporated. *MLNE* is very fast (with reliable estimates being achieved in seconds to minutes) which allowed extensive computer simulations and comparison of

its performance to that of the moment-based estimators (Wang 2001). This demonstrated that *MLNE* is as good or better than the moment-based estimators under all conditions and, in particular, it is far less biased when using loci with many alleles at low frequencies (such as commonly found at microsatellite loci). *MLNE* also includes a facility allowing the user to test for differences in N_e during the intervals defined by three or more samples.

The pseudolikelihood method may yield an approximation to the likelihood curve that overestimates the confidence in the estimate. Wang (2001) investigated this, using simulation, and found that the confidence intervals contained the true value of N_e about 95% of the time, as they should. However, Tallmon *et al*. (2004) found that under some conditions, the confidence intervals provided by *MLNE* included the true value of N_e only 65% of the time. The confidence intervals were less reliable for larger sample sizes and for a small number of generations separating the samples.

Coalescent methods

A different likelihood model for temporally-spaced samples may be derived by considering the coalescent process. Berthier *et al*. (2002) were the first to apply the coalescent model in a temporal method framework. In their model, the genes in the second temporal sample are assumed to be descended, without mutation, from ancestral genes that existed in the population at the time that the first sample was taken. Under such a model, the relationship between N_e and the degree of allele frequency difference between the two samples depends on the fact that the rate of coalescence increases with decreasing N_e, and can be described as follows: if N_e is large, then the number of genes ancestral to sample 2 that are extant at the time of the first sample will be close to the

actual number of genes in the second sample (*i.e*. there will have been few coalescences) so the allele frequencies in the second sample will be determined by a large “sample" of ancestors and will not differ greatly from the allele frequencies in the first sample. However, if N_e is small, then many coalescences will have occurred, so the second sample will have descended from few ancestors, and the allele frequencies of the descendants could be quite different from those of the first sample. Calculating the likelihood for this model involves a sum over the unknown allelic types possessed by the genes ancestral to those in the second sample. With multiallelic loci, this sum may be effectively intractable, so Berthier *et al*. (2002) approximate the likelihood using a scheme involving importance sampling imbedded within MCMC. Their method is implemented in the computer program *TM3*.

Beaumont (2003) extended the model of Berthier *et al*. (2002) to multiple samples taken in time, allowed a way to test for population growth or decline, and described and implemented several computational improvements. The modifications are incorporated in the computer program *TMVP*. Both *TM3* and *TMVP* require hours of computer time to collect a sufficiently large Monte Carlo sample. Anderson (2005) used an importance sampling scheme to compute the two-sample likelihood of Berthier *et al*. (2002) in a matter of seconds. This method is implemented in the computer program *CoNe*. It must be stressed that although these methods are based on the coalescent process, they still estimate the N_e of the population during the interval between the two samples. They do not estimate the long-term or historical N_e as do the single-sample, coalescent-based, likelihood methods.

Berthier *et al*. (2002) evaluated their estimator on a collection of simulated data sets, and found that it is less biased than the *F*-statistic-based estimators, with a slight downward bias when a small number of generations separate the samples. Tallmon *et al*. (2004) reported the same finding with Beaumont's (2003) *TMVP* method, showing that its estimates are more biased than those of *MLNE*. More problematic than the bias, however, is that the 95% confidence intervals computed by *TMVP* appear to be inaccurate. In some of the simulations reported by Tallmon *et al*. (2004) the true value of N_e fell above the 95% confidence interval well over 50% of the time (instead of the 2.5% expected).

Despite these caveats, the coalescent-based temporal method is valuable in a number of contexts. Most notably, the coalescent model is more natural than the hidden Markov-chain / Wright-Fisher model, implemented in MCLEEPS and MLNE, for samples drawn from a population lacking discrete generations. Furthermore, when very long time periods separate the samples, the coalescent method may be more computationally efficient because it is not necessary to explicitly account for each generation with a matrix multiplication (as in *MLNE*), although it may no longer be reasonable to ignore mutation. And finally, though it has not yet been pursued, the mathematical framework developed for the coalescent-based temporal method could be used to directly estimate the average inbreeding coefficient, *F*, within a population. Such an approach would provide an exact version of the procedure proposed by Laval *et al*. (2003) which relies on an approximation that holds only for small values of *F*.

Approximate Bayesian computation methods

An alternative computational approach to estimation of effective population size was described by Tallmon *et al*. (2004). This method uses simulation under the Wright-Fisher, hidden Markov-chain model to make inferences about N_e. It is based on approximate Bayesian computation (ABC)—a technique related to the idea of rejection sampling for population-genetic inference (Beaumont et al. 2002; Di Rienzo et al. 1994). The ABC approach, termed the *SummStat* method, provides a way to use summary statistics computed from molecular data to infer likely values of N_e. This avoids the computational burden of calculating the exact likelihood and, by using multiple summary statistics simultaneously, the quality of the inference may be comparable to that achieved using the full likelihood.

Operationally, the *SummStat* method produces an estimator as follows. First, several summary statistics are computed based on the observed, temporally-spaced samples. In their example, Tallmon *et al*. (2004) used four statistics (whose observed values we denote by the vector $\boldsymbol{s}_{obs}$): *i*) the estimated coancestry coefficient between the samples, *ii*) the mean, across loci, of the change in the number of alleles observed between the samples, *iii*) the change between samples of the within-sample gene diversity, and *iv*) the total expected heterozygosity between samples. Second, a large number (50,000 in their example) of N_e values are drawn from a uniform distribution between 4 and 400. For each simulated value $N_e^{(i)}$, genetic data sets—temporally spaced as the observed data—are sampled from a simulated Wright-Fisher population of size $N_e^{(i)}$, and the four summary statistics, denoted $\boldsymbol{s}^{(i)}$, are computed from the simulated genetic data. If $\boldsymbol{s}^{(i)}$ is

similar to $\boldsymbol{s}_{obs}$ then the value of $N_e^{(i)}$ is included in a sample of N_e values, which is used [in conjunction with the weighting scheme discussed in Beaumont *et al*. (2002)] to estimate the true value of N_e. In effect, values of N_e which give rise to simulated data sets that have summary statistics close to the observed data set are taken to be a sample from the approximate posterior distribution of N_e, and that sample is used to make approximate Bayesian inference about N_e.

Tallmon *et al*. (2004) compare the performance of their *SummStat* method to that of two other likelihood-based methods (*MLNE* and *TMVP*). They find that for most simulated scenarios the *SummStat* estimator has lower bias than the others and an intermediate variance. The 95% confidence intervals for N_e computed by the *SummStat* method also seem to be more accurate than for the other two methods. Unfortunately, there seems to be no user-friendly software implementing the *SummStat* method.

Coalescent with mutation methods

A separate line of research has exploited temporally-spaced samples to estimate effective population size and mutation rate within a coalescent framework but in the presence of mutation (Drummond *et al*. 2001; Drummond *et al*. 2002; Rodrigo & Felsenstein 1999). In this work, the data are samples of DNA sequences taken at different times. Since these models allow for mutation, and because they use sequence data, the underlying likelihood function is more complex than that used in *TM3* and *TMVP*. Drummond *et al*. (2003) review the methods and software available for analyzing such temporally-spaced sequence data. These methods *do not* estimate the N_e of the population during the interval

between the samples. Rather, they are more closely related to the single-sample estimators of N_e which estimate $\Theta = 4N_e\mu$ (see above). The inclusion of temporally-spaced samples in these models allows independent estimation of N_e and μ, rather than estimation of the composite parameter Θ. Accordingly, the N_e being estimated by these methods is the long-term, or historical N_e, over the entire coalescent history of the samples. In order to be informative about N_e and μ separately, the time period separating the samples must represent a substantial portion of the total coalescent history of the samples, and the mutation rate of the sequences must be high enough that a number of mutations will have accumulated in lineages between the different sampling episodes. For these reasons, such methods were originally directed toward data from viruses sampled at different times within a single infected patient. However, ancient DNA, such as that from sub-fossils and museum specimens, is increasingly available and amenable to such analysis (Drummond *et al*. 2003).

A number of studies have appeared recently that deal with specific issues encountered in applying the temporal method to realistic scenarios, particularly those involving salmonids. Waples and Yokota (2006) investigate the effects of various types of overlapping-generation demographies on the temporal method. A potential difficulty has been pointed out by Araki et al. (2007), who indicate that biases may arise when estimating the N_e of populations in which one component of the population suffers a lower survival rate than the other, as might occur in artificial propagation programs.

Changes in population size

Frequently, investigators are more interested in demographic trends than in actual estimates of population size. This is particularly true in conservation biology, where it can be important to understand whether current status is indicative of long-term, historic patterns or if it is primarily due to recent anthropogenic effects. Increases in population size can result in changes to management policy that have significant economic or cultural consequences for human activities. Conversely, rapid reductions in population size, or bottlenecks, can reduce evolutionary potential and increase extinction risk (Frankham 1995; Lande 1988). It is thus important to identify them so that appropriate measures can be taken. Moreover, substantial reductions in N_e can occur without concurrent changes in census size or in ways that are hard to detect. Luikart *et al*. (1998b) describe several such cryptic bottlenecks.

Change in size—multiple sample methods

When the trend in number of individuals is of primary interest, traditional N_c estimation methods can be used, if the population can be sampled at multiple points in time. Such monitoring can involve so-called exhaustive methods, or use genetic or other tags in fractional mark/recapture methods. As with all abundance estimates, the temporal scale of relevance for trend estimates includes only the generations bracketed by sampling. Genetic monitoring techniques can also infer changes in N_e between sampling points by comparing estimates of genetic variation (Nei *et al*. 1975), although such methods may not have much power (Luikart *et al*. 1998b). The temporal method can be used to infer changes in N_e from one time interval to the next when genetic data from more than two

time points are available (Beaumont 2003; Pollak 1983; Wang 2001; Waples 1989). It is occasionally possible to obtain genetic data from museum, or other archived, tissues (Nielsen *et al*. 1997; Nielsen *et al*. 1999; Thomas *et al*. 1990), which can provide inference about N_e many generations into the past and, occasionally, allow comparisons between contemporary and effective size for some historical interval.

Change in size—single sample methods

A more difficult problem is inference about changes in population size from a single sample in time. It is virtually impossible to detect and evaluate changes in N_c with a single sample in time, although data on age structure or density dependence in other, related, populations may provide some inference. However, several methods for studying change in N_e using genetic data from a single sample in time have been described.

An early such method uses the distribution of pairwise differences in a population sample of DNA sequences (Rogers & Harpending 1992; Slatkin & Hudson 1991) to infer change in N_e. Populations that have been stable are expected to have frequency distributions of the number of pairwise differences that are shaped differently than those from populations that have increased in size. The pairwise differences method has been applied extensively to mtDNA to detect size changes over 1000s of generations. It has also been used to estimate the timing and magnitude of such changes. The method, and in particular the parameter estimation elements, has been roundly criticized (e.g. Bertorelle & Slatkin 1995), because there are multiple scenarios that can yield the same observed distribution shape. Schneider and Excoffier (1999) extend the pairwise differences method in several ways, including incorporation of a more realistic mutation model for

the DNA sequences to which it is most commonly applied. A number of additional methods which exploit the expected patterns of a summary statistic due to historical population growth have been described. These methods test for a past episode of population expansion, but do not attempt to estimate population growth rates (Laan & Pääbo 1997; Kimmel et al. 1998; Reich et al. 1999; Gonser et al. 2000).

Coalescent methods have been used to obtain maximum likelihood estimates of population growth rates from a single sample in time, with Griffiths & Tavaré (1994b) and Kuhner *et al*. (1998) considering an exponential growth model and Beaumont (1999) considering several growth models. These methods make use of all aspects of the data that are affected by the population's current and historical sizes and thus provide estimates of growth rates that represent the entire coalescent history of the genetic sample. To the extent that the inference derived from such methods is the result of the topology of the underlying genealogy, they should be useful for detecting long-term, gradual changes in population size, as changes in population size only have an appreciable effect on the coalescent when the new population size prevails over a time, measured in number of generations, that is of the same order of magnitude as the new population size itself (Nordborg & Tavaré 2002). Effective size estimates over more than one generation are expressed as the harmonic mean over that interval, meaning that small sizes dominate the estimate. Single-sample estimates can thus be problematic when N_e has been severely reduced in the recent past, thus dominating the estimate, and the goal is to understand prehistoric N_e.

Many investigators, particularly in conservation biology, are interested in recent reductions in size, frequently from anthropogenic causes. Such reductions may not be

detected by likelihood methods that estimate long-term rates of population growth or decline. For such situations, several summary statistic-based methods for detecting recent reductions in effective size using a single genetic sample have been developed. These methods exploit the transient effects of strong genetic drift on the genetic characteristics of populations. Cornuet and Luikart (1996) describe the utility of the differential rate of post-reduction decline of two measures of genetic diversity - number of alleles and heterozygosity - in detecting recent reductions in N_e. They demonstrate how a transient excess in the heterozygosity, relative to that expected in a stable population with the same number of alleles, can be used to detect recent bottlenecks. Garza and Williamson (2001) describe a similar method for detecting recent bottlenecks, which also exploits the differential rate of change of two measures of genetic variation for microsatellite loci - number of alleles and range in allele size - following a reduction in N_e. The ratio of the two measures is the test statistic. Both methods use features of the allele frequency distributions expected in populations with recent changes in N_e and are highly dependent upon assumptions about the mutation model. While both methods only detect bottlenecks that occurred recently, it appears that the ratio method can detect bottlenecks occurring further in the past (Garza & Williamson 2001).

Luikart *et al*. (1998a) also propose a graphical method for detecting recent reductions in effective size in which the number of alleles in a sample of loci is plotted with respect to their frequency in the population, using pre-determined frequency intervals (in their example, 10%). If the rare allele classes have fewer alleles present than the higher frequency ones, then one concludes that a population has been reduced in effective size, because genetic drift generally removes rarer alleles first. A potential problem with this

qualitative method is that the frequency intervals must be arbitrarily defined, and changes in the size of the intervals can potentially give different shaped histograms that may be interpreted differently.

Additional considerations

Most of the differences in definitions and estimators of population size can perhaps best be understood in the context of the Wright-Fisher model. If a real population satisfied all of the assumptions of the model and was exactly an ideal population, then all definitions of population size would be the same. However, real populations frequently depart from assumptions of the Wright-Fisher model (Crow & Kimura 1970; Ewens 1982). Much theoretical work has been done to predict the effects of such departures on N_e (e.g. Caballero 1994; Nunney 1993). However, little work has been done to incorporate such theoretic results into procedures for estimating N_e using molecular data. This is likely due to the complicated nature of the models that would be necessary and the difficulty in evaluating and estimating departures from the model in many natural populations.

Many differences in population size estimates and their interpretation can also be attributed to issues of scale. For estimation of N_c using mark/recapture methods, the temporal scale of relevance is the generation of sampling for populations with non-overlapping generations, or the set of generations represented by the sampled individuals in an age-structured population. However, such estimates can also provide limited, asymmetric information about population size in the generations immediately preceding sampling, because maximum population growth rate sets a lower bound on size for these preceding generations. The spatial scale of relevance for such abundance estimates is

determined by the sampling scheme and, to a lesser extent, dispersal rate and distance. It should be noted that undetected sampling of individuals from two or more populations that are not exchanging genes can lead to substantial overestimates of abundance. For populations of conservation concern, such errors could have large effects on extinction probabilities estimated in population viability analyses.

In many cases, investigators may be interested in estimating N_e of multiple subpopulations that are exchanging genes but are not panmictic. Such "global" effective size estimates can vary dramatically depending upon the details of the migration matrix, variance in reproductive success among subpopulations, and the distribution of subpopulation sizes. Nunney (1999) and Waples (2002) have detailed how N_e estimates of a subdivided population can be greater or less than the sum of subpopulation effective sizes. A metapopulation is simply a special case of a subdivided population where extinction of subpopulations is allowed (Barton & Whitlock 1997). As noted above, the use of assignment methods to identify migrants may help to estimate elements of the migration matrix between subpopulations. Alternatively, Beerli & Felsenstein (1999, 2001) describe likelihood methods for a single genetic sample, and Wang and Whitlock (2003) describe both moment- and likelihood-based methods for temporal samples, which jointly estimate N_e of, and migration between, multiple subpopulations comprising a subdivided population. Fraser et al. (2007) report on a comparison of a number of different estimators of N_e and migration rate in two salmonid species known to have contrasting population structures. Although the true values of the parameters being estimated in these populations were not known, comparison of results between the different species is informative. They concluded that if one suspects migration may be

affecting the study population, N_e should be estimated both with and without the assumption that there is migration; however, they identify a pressing need for new methods of jointly estimating N_e and migration rate which derive from more flexible population models. In a meta-analysis of 83 studies done since 1995, Palstra and Ruzzante (2008) report that estimated N_e values can be heavily influenced by the occurrence of gene flow. Again, this argues for the importance of accounting for gene flow when estimating N_e in populations that may not be completely isolated.

Many populations, particularly those of conservation concern, are unlikely to be in mutation-drift equilibrium. In such cases, estimates of long-term and contemporary effective size may differ markedly. Further, in populations that have recently undergone a population decline, the contemporary inbreeding effective size can be larger than the contemporary variance and eigenvalue effective sizes (Waples 2002). However, it is important to point out that this is a transient phenomenon, due to changing population size and the fact that inbreeding effective size is related to parental or grandparental generations while the variance effective size is related to the progeny generation (Crow and Kimura 1970), and not that they are inherently reflective of a different portion of a population's history. Indeed, Pollak (2002) has shown that the three common effective sizes are roughly equivalent, even when population size changes, when viewed over several generations. Additionally, over temporal scales associated with mutation-drift or migration-drift equilibrium, Whitlock & Barton (1997) show that most of the different definitions of effective size are equivalent, even with spatial subdivision.

The scale of reference for N_e estimates is also affected by the life stage at which individuals are sampled. Some disagreement among early methods and authors occurred

as a result of when samples were drawn relative to when in the organism's life-history genetic drift occurred. Two different sampling "plans" for the temporal method identified by Nei & Tajima (1981b) have substantial consequences for estimates of effective size. In Plan 1, individuals are sampled once they have reached reproductive maturity. In Plan 2, individuals are sampled as juveniles, before the typical periods of high mortality that result in genetic drift of allele frequencies. Both of these sampling plans can be treated within a common *F*-statistics framework with minor adjustments (Waples 1989), with attention paid to the specific generations in which the estimates apply. The newer likelihood and Bayesian methods make assumptions that generally correspond to one sampling plan or the other. However, these methods should be able to be modified to explicitly incorporate different sampling schemes.

The relationship of different definitions of N_e and their time scales of reference have given rise to some lively exchanges in the literature. We propose here that much disagreement can be avoided by careful consideration of both the particular definition of N_e and the time interval over which it is believed to apply to a population. Fortunately, the interpretation of effective size can be made easier in the future if population biologists/molecular ecologists adhere to a few simple guidelines.

1. When reporting an effective size for a natural population, always state the time period over which it is believed to prevail. Natural populations are dynamic entities, with census sizes or habitat ranges that may fluctuate dramatically over time. Correspondingly, the effective size of a population may also change over time, and it makes little sense to use the phrase "effective size" with no qualifying details.

2. Different genetically-based methods for estimating effective size apply to specific time periods and time scales. However, estimators should not be confused with definitions. Note that estimators are sometimes associated with different definitions of effective sizes, and care should be taken to not blur the distinction.

3. None of the three main effective sizes - inbreeding, variance, or eigenvalue - are intrinsically associated with a particular time scale. However, the different population-genetic measures associated with these effective sizes are affected by deviations from the assumptions of an ideal population on slightly different time scales. For example, the inbreeding effective size is related to the parental or grandparental generation and the variance effective size is related to the progeny (sampled) generation. So when estimating contemporary effective size, care must be taken to specify exactly for which generation the estimate applies.

We recommend that these three guidelines be kept in mind when deriving, interpreting and reporting estimates of effective size of natural populations, whether those estimates derive from genetic data or demographic considerations. Such practice should reduce confusion over future estimates and interpretation of effective population size.

Acknowledgements

We thank Louis Bernatchez, Philip England, David Tallmon and Robin Waples for helpful discussions and sharing manuscripts with us before publication. Two anonymous reviewers also provided valuable suggestions.

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Figure 1. Temporal scales of reference for Ne estimation. Illustration of the temporal scale of reference for effective size estimation methods. The interval depicted for the temporal methods is arbitrary and typically does not include the generation of the second sample. The dashed line indicates the penultimate or last several generations in the case of the heterozygosity excess and disequilbrium methods, respectively.

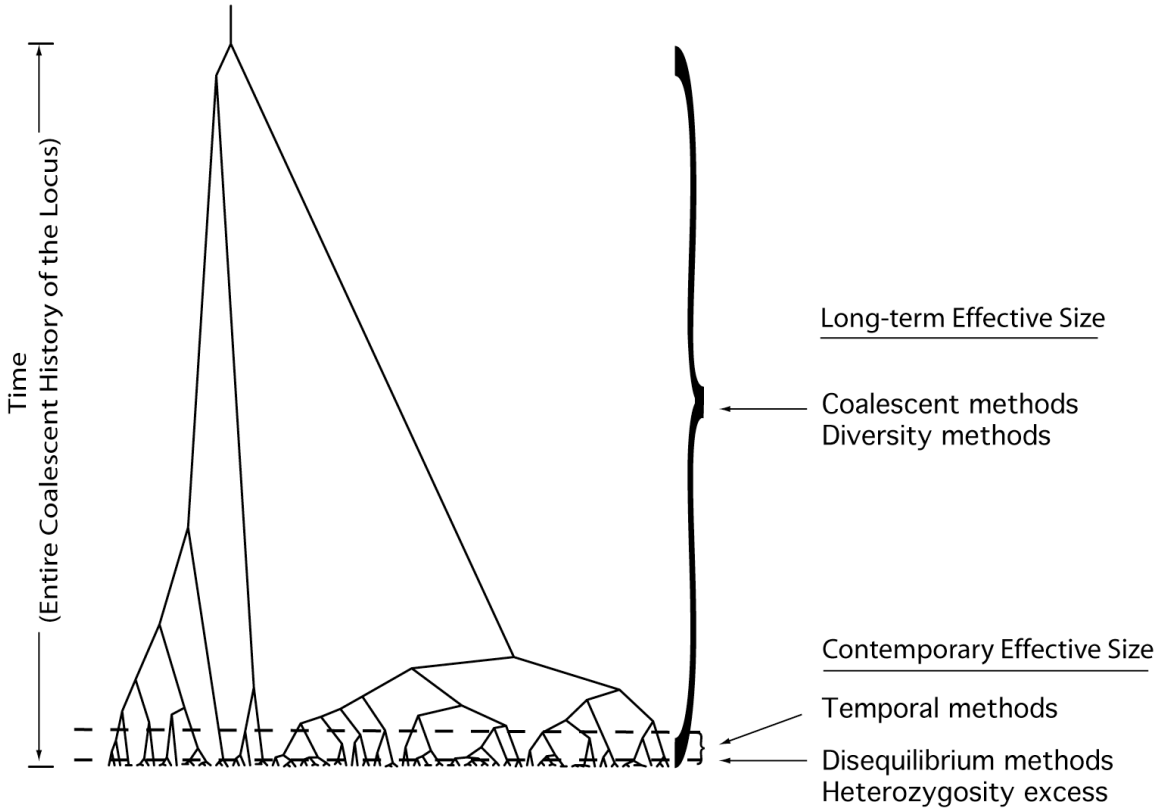

Table 1. Programs available for estimation of effective population size and related quanitities

Method Citation	Software[a]	Temporal Scale[b]	Genetic Markers[c]	Run Times[d]	Statistical Method[e]	Computation Method[f]	Simulation Testing[g]	Performance[h]
NE ESTIMATION								
Mutation-drift equilibrium								
Kuhner et al. (1995)	COALESCE[i]	LT	S,M,A,B	3-4	ML	MCMC	M	U
Nielsen (1997)	MISAT	LT	M	3-4	ML	IS	M	H[j]
Wilson & Balding (1998)	Micsat	LT	M	3-4	B	MCMC	M	---
Wilson et al (2003)	Batwing	LT	M,B	3-4	B	MCMC	M	N
Xu and Fu (2004)	----[k]	LT	M	1	MM	D	M,R[l]	U
Griffiths & Tavaré (1994a)	Genetree	LT	S	2-4	ML	IS	S[m]	---
Drummond et al. (2002)	BEAST	LT	S	2-4	B	MCMC	M	A
Tallmon et al. (2008)	ONeSAMP	LT	M	2	B	RS	S	---

[a] Software for implementing the method. Most software packages may be easily found by searching the Internet for the software name plus "genetics" or "estimation," etc. If not, the URL is given in a footnote. Note that the software is not always written by the author of the original method, especially for the earlier methods.

[b] LT= "long term" or the scale of the entire coalescent history of the sample. C = contemporary. M= between LT and C.

[c] A=allozymes; B=binary markers like SNPs; M=microsatellites; S=sequence data; D=dominant markers such as AFLPs.

[d] Approximate scale of run times: 1=seconds; 2=minutes; 3=hours; 4=days.

[e] B=Bayesian; ML=maximum likelihood; MM=method of moments; PL=pseudolikelihood; SS=summary statistic.

[f] D=deterministic; IS=importance sampling; MCMC=Markov chain Monte Carlo (includes simulated annealing); RS=Rejection sampling---this includes approximate Bayesian computation (Beaumont et al 2002).

[g] Extent to which the method has been tested on simulated data. S=small sets of simulations to verify computational method works; M=multiple simulations to assess average behavior; R=robustness testing: method has been applied to data not simulated under the model used for inference. When the testing was presented in the original paper on the method, no citation is given. If the testing is presented in a separate paper then the citation is given in a footnote.

[h] Performance of the estimator if reported. For point estimator: U=bias is inconsequential; h=small upward bias; H=substantial upward bias; l=small downward bias; L=substantial downward bias. For interval estimators or hypothesis tests: A=interval estimators or hypothesis tests are accurate, i.e. the true value falls within the interval as frequently as expected, or the reported type I error rate is accurate. N=true value falls within interval less often than expected; C=true value falls within interval more often than expected (intervals are conservative).

[i] Now a component of the LAMARC package.

[j] Xu and Fu (2004)

[k] Unnamed software available upon request from the authors.

[l] When data are simulated under a stepwise mutation model allowing for steps > 1, the estimator becomes upwardly biased.

[m] A small number of simulations were done by Stephens & Donnelly (2000), assessing the Monte Carlo variance of early versions of the Griffiths & Tavaré (1994a) importance sampling scheme.

Table 1. Programs available for estimation of effective population size and related quanitities (continued)

	Method Citation	Software	Temporal Scale	Genetic Markers	Run Times	Statistical Method	Computation Method	Simulation Testing	Performance
	Kuhner (2006)	LAMARC[a]	LT	S,M,A,B	3-4	ML,B	MCMC	M[b]	U
Gene Identity, LD, and Sibship Analysis									
	Hill (1981)	NeEstimator[c]	C	M,A,B	1	MM	D	M[d]	h
	Vitalis & Couvet (2001b)	Estim 1.0[e]	M	M,A,B	1	MM	D	M,R[f]	l
	Pudovkin et al. (1996)	NeEstimator	C	M,A,B	1	MM	D	M	h[g]
	Waples (2006)	LDNe[h]	C	M,A,B	1	MM	D	M,R[i]	U[j]
	Wang (2009)	Colony	C	M,A,B,D	2-3	ML	MCMC	M,R	U,N,C[k]
Temporal Methods									
	Krimbas & Tsakas (1971)[l]	NeEstimator	C	M,A,B	1	MM	D	M	h[m]
	Williamson & Slatkin (1999)	MLNE[n]	C	M,A,B,D	1-2	ML	D	M	h
	Anderson et al. (2000)	MCLEEPS	C	M,A,B	2-3	ML	IS	S	---
	Wang (2001)	MLNE	C	M,A,B	1-2	PL	D	M	h

[a] This program brings together COALESCE, FLUCTUATE, and RECOMBINE, into a single program that can handle many different types of evolutionary/demographic processes and which offers the option of Bayesian inference.

[b] Extensive simulation results are reported in Kuhner & Smith (2007).

[c] Peel et al. (2004).

[d] Ovenden et al. (2004) report this estimator is biased upward when true N_e is much larger than sample size.

[e] Presented in the program note of Vitalis & Couvet (2001a)

[f] The original authors report on robustness to departures from the infinite island model and mutation-drift equilibrium.

[g] Luikart & Cornuet (1999) found the estimator to be only slightly biased upward, but also report that if the effective number of breeders is >10, then a very large number of loci and individuals must be sampled to achieve useful precision of the estimate.

[h] Presented in the program note of Waples and Do (2008).

[i] Data were simulated from populations that were not Wright-Fisher populations, but for which inbreeding N_e could be computed.

[j] However, see Russell & Fewster (2009).

[k] Depending on simulation conditions, CIs were either conservative or non-conservative.

[l] Other authors who refined the method include Nei & Tajima (1981b) and Pollak (1983) and Waples (1989).

[m] Bias is slight, but increases as the number of low frequency alleles increases in the sample.

[n] For diallelic loci, the method of Wang (2001) implemented in MLNE is very similar to that of Williamson & Slatkin (1999), albeit with some computational tricks to improve speed.

Table 1. Programs available for estimation of effective population size and related quanitities (continued)

Method Citation	Software	Temporal Scale	Genetic Markers	Run Times	Statistical Method	Computation Method	Simulation Testing	Performance
Berthier et al. (2002)	TM3	C	M,A,B	2-3	B	MCMC	M	l[a]
Beaumont (2003)	TMVP	C	M,A,B	2-3	B	MCMC	S	---
Tallmon et al. (2004)	----[b]	C	M,A,B	2	B	RS	M	h
Anderson (2005)	CoNe	C	M,A,B	1-2	ML	IS	M,R[c]	h
POPULATION GROWTH RATE ESTIMATION								
Kuhner et al. (1998)	FLUCTUATE[d]	LT	S,M,A,B	3-4	ML	MCMC	M	H/l[e]
Griffiths and Tavaré (1994b)	Genetree	LT	S	3-4	ML	IS	---	---
Wang (2001)	MLNE	C	M,A,B	1-2	PL	D	M	L/H[f]
DETECTION OF CHANGES IN POPULATION SIZE								
Schneider & Excoffier (1999)	Arlequin	LT	S	1	SS	D	M	A
Beaumont (1999)	MSVAR[g]	M	M	3	B	MCMC	S	---
Cornuet & Luikart (1996)	BOTTLENECK	C	M,A,B	2	SS	RS	M	---[h]
Garza and Williamson (2001)	M_p_val	C	M	1-2	SS	RS	M	

[a] The median value of the estimates was below the true value. The authors did not report behavior of the mean value of the estimates. TM3's estimator is probably biased slightly upward, as it is for CoNe (Anderson 2005).

[b] There is apparently no user-friendly implementation of this method.

[c] Simulations were done with mutation rate greater than zero, counter to the assumption of the method.

[d] Now a program in the LAMARC package.

[e] Growth rate estimates were almost universally, strongly biased upward, but estimates of N_e were slightly biased downward using a model that included non-zero growth rate.

[f] For moderate to large initial population sizes, the growth rate is underestimated and the initial effective population size is overestimated.

[g] Available at: http://www.rubic.rdg.ac.uk/~mab/software.html.

[h] Test for occurrence of population size changes was found to be sensitive to the true mutation model of the markers (for example it performs poorly when the data come from a single step mutation model).

Table 1. Programs available for estimation of effective population size and related quanitities (continued)

Method Citation	Software	Temporal Scale	Genetic Markers	Run Times	Statistical Method	Computation Method	Simulation Testing	Performance
JOINT ESTIMATION OF NE AND MIGRATION RATE								
Beerli & Felsenstein (1999, 2001)	MIGRATE[a]	LT	M,S	4	ML[b]	MCMC	M,R[c]	l[d]
Wang & Whitlock (2003)	MLNE	C	M,A,B	1-2	MM,ML	D,PL	M,R	l,u[e]

[a] Now a program in the LAMARC package.

[b] See Beerli (2006) for discussion of a Bayesian version of MIGRATE.

[c] Beerli (2004) explores the effect of unsampled populations on the estimates.

[d] Beerli & Felsenstein (2001) report that in their simulations, the estimates, on average, were a little smaller than the true values of the parameters; however, they point out that on analytical grounds, the expectation of the estimator might not even be finite, so discussing whether the estimator is biased may not be appropriate.

[e] Estimates of migration rate become biased downward and of N_e become biased upward as the scaled time (number of generations divided by $2N_e$) between samples increases above about 0.08.

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