

GENETIC APPROACHES FOR ESTIMATING THE EFFECTIVE SIZE OF POPULATIONS

PAUL LEBERG,¹ Department of Biology, University of Louisiana, Lafayette, LA 70503, USA

Abstract: The effective population size (N_e) provides information on how fast genetic variation is being lost, or relatedness is increasing, in a population of interest. This parameter is often considered to be related to population viability. Genetic approaches offer several avenues for estimating N_e ; recent developments have helped relax assumptions of closed populations and stable population sizes that have hindered the estimation of N_e in many wildlife populations. The most promising areas of development are in the assessment of temporal changes in genetic composition for the estimation of contemporary N_e and the application of coalescent theory for estimation of historical N_e . Application of most estimators still requires making some questionable assumptions about wildlife populations. In general, these methods will be most beneficial when used in conjunction with current and historical demographic information.

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As molecular tools have made quantification of genetic variation easier, there have been hundreds of studies assessing the genetic characteristics of wildlife species. One of the most discussed, yet least measured, genetic parameters of wildlife populations is the effective population size (N_e ; Wright 1931). In cases where N_e is small, a population might rapidly lose genetic variation or accumulate relatedness (Crow and Denniston 1988; Frankham 1995*a,b*; Schwartz et al. 1998).

Knowledge of N_e is critical in managing threatened species, as well as captive populations, because it provides information on how fast a population is losing genetic variation (Fig. 1). Population fragmentation and isolation, harvest strategies, and translocations are thought to have considerable impact on N_e (Ryman et al. 1981, Leberg 1990*a*, Miller and Waits 2003). Reductions in N_e are related to reduced population viability (Lande and Barrowclough 1987, Soulé 1987, Leberg 1990*b*, Newman and Pilson 1997). Given the potential association between N_e and the probability of extinction, estimates of N_e might be used to assess the vulnerability of taxa (Mace and Lande 1991). Additionally, comparison of historic to contemporary N_e could be used to assess whether a population is declining (Funk et al. 1999). Thus, estimation of N_e can provide a better understanding of the implications of management activities and environmental changes on the genetic future of populations (Lande and Barrowclough 1987, Simberloff 1988).

Traditionally, N_e has been very hard to measure, but that situation is changing (Schwartz et al. 1998). My objective is to provide an introduction to the use of genetic approaches for estimating N_e that might be useful for the study of wildlife populations. Since this topic was last reviewed in relation to wildlife (Frankham 1995*b*, Schwartz et al. 1998), new forms of estimators have been developed. Although it is possible to estimate N_e with demographic models (Harris and Allendorf 1989, Nunney and Elam 1994), I focus on genetic approaches, which have seen a revolution in new developments over the last decade. Furthermore, estimates of N_e based upon genetic approaches are often smaller than predicted by the demographic models (Frankham 1995*b*). It appears that the lower estimates of N_e obtained from genetic estimators reflect population fluctuations and temporal variation in reproductive success that may be missed in demographic models. Given the rapid development of genetic approaches used to estimate N_e , it is reasonable to assume that my review will soon be out-of-date. However, hopefully this introduction to the literature will provide useful guidance to wildlife biologists with regard to what approaches are available and the types of questions they can help resolve. For a more detailed treatment of many of the estimators, see Beaumont (2003*a*).

WHAT IS EFFECTIVE POPULATION SIZE?

Using classical population genetics theory, it is possible to predict how rapidly genetic diversity should be lost from a population of a given size (Crow and Kimura 1970). For example, average heterozygosity decreases due to increased com-

¹ E-mail: leberg@louisiana.edu

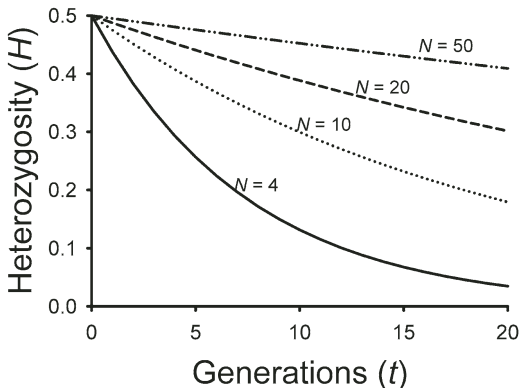


Fig. 1. Influence of number of generations and population size (N) on loss of genetic diversity, as measured by heterozygosity.

mon ancestry at a rate of $1/2N$ per generation (Fig. 1). Ideally, an estimate of the number of individuals of breeding age (hereafter the census population size, N_c), could be substituted for N in this relationship, making it possible to determine how rapidly the population is becoming less heterozygous. However, theoretical predictions of rates of loss of variation usually assume that every individual in the population has an equal probability of successful reproduction and that self-fertilization is possible. These assumptions usually do not hold for wildlife populations. Individuals may have different probabilities of reproduction because of age or limitations on breeding territories. Most wildlife species only reproduce sexually, and breeding structure or unequal sex-ratios can create differences among individuals in their probabilities of transmitting genes to the next generation. This simple model for loss of heterozygosity also assumes that the population size is stable; that generations do not overlap; and that there is no selection, mutation, or migration. When any of these assumptions are violated, the rate of loss of heterozygosity could differ from the theoretical expectations obtained by treating N_c as the size of the population.

One way of improving predictions of the loss of genetic diversity is to estimate N_e . The N_e of a population is the number of individuals in an ideal population that would lose genetic variation at the same rate as the actual population (Crow and Denniston 1988, Schwartz et al. 1998). In an ideal population, all individuals have an equal chance of being the parents of any progeny making up the next generation. This ideal population is generally considered to be temporally stable and free of mutation, natural selection, and migration. No

wildlife population perfectly fits this description. Thus, it is expected that N_e will differ from N_c . Usually $N_e < N_c$, but this is not always the case (Crandall et al. 1999). The many factors that can cause $N_e \neq N_c$, including unequal sex ratios, high variance in reproductive success among individuals, nonrandom mating, mating systems, multiple paternity, gene flow, overlapping generations, and temporal fluctuations in population size, have been discussed numerous times (Crow and Kimura 1970; Chesser 1991a,b; Sugg and Chesser 1993; Nunney 1999; Hedrick 2000; Oyler-McCance and Leberg 2005). I focus on how to use genetic approaches to estimate N_e in wildlife populations.

One difficulty in discussing N_e is that it has been applied to many different components of genetic diversity (Crow and Denniston 1988). There are 2 commonly estimated measures of N_e . Variance effective size (N_{eV}) is the size of an ideal population experiencing drift (random changes in allele frequencies) at the same rate as the actual population. Inbreeding effective size (N_{eI}) is the size of an ideal population losing heterozygosity, due to increased relatedness, at the same rate as the actual population. Typically, N_{eI} and N_{eV} are not discussed in detail in most reviews because for stable, large populations N_{eI} and N_{eV} are similar. Unfortunately, the sizes of many of the populations of conservation interest are not stable; when populations are growing or shrinking N_{eI} and N_{eV} can greatly differ (Crandall et al. 1999). Furthermore, many wildlife populations exhibit social structure. The degree of genetic exchange in socially structured populations can greatly influence the number of generations it takes for N_{eI} and N_{eV} to approach asymptotic levels where they become identical (Chesser et al. 1993). Thus, the type of N_e being estimated needs to be clearly stated (Neigel 1996, Crandall et al. 1999). There are additional forms of N_e (Ewens 1982, Crow and Denniston 1988, Neigel 1996, Crandall et al. 1999). However, most genetic approaches attempt to estimate N_{eI} or N_{eV} , as do most demographic studies (Crow and Denniston 1988). I focus primarily on these 2 most commonly discussed forms of N_e ; however, I will also discuss a few estimators of alternate forms of N_e .

Understanding the differences between N_{eI} and N_{eV} is necessary to consider how management activities or environmental changes affect genetic diversity. A rapid decrease in population size is likely to cause a concurrent decrease in N_{eV} , resulting in more random genetic change (Kimura and Crow 1963). Associated with this increased drift is the loss

of alleles from populations, which potentially reduces the ability of populations to respond to environmental change through natural selection (Allendorf and Leary 1986, Frankham 1995a). Although a rapid decrease in population size will also decrease N_e , it is likely to remain large in the generations immediately following a bottleneck. In other words, a population that has recently declined in number will accumulate inbreeding more slowly than it experiences drift. If a population remains small, N_{el} will eventually be reduced to N_{eV} .

Differences in the measures of N_e in response to changes in N_c are also important in assessing the genetic effects of management activities that encourage population growth. As a population recovers from a small size, N_{eV} will increase with N_c ; however, it will take much longer for N_{el} to recover (Kimura and Crow 1963). Relatedness that has accumulated in a population when it was small will not be reduced rapidly by obtaining a large N_c but additional drift and loss of allelic variation will be minimized.

When discussing estimates of N_{eV} and N_{el} it is also important to note that these estimates are often made over different time scales; Crandall et al. (1999) refer to these as contemporary and historical N_e . Often N_{eV} is measured by taking 2 temporally spaced samples. In this case, the estimate of N_{eV} is for the period between these samples (Waples 1989) and is considered to be a measure of contemporary N_e (Turner et al. 2002). On the other hand, N_{el} is often measured using coalescent-based models; such approaches reflect the average N_e over many generations (Neigel 1996, Schwartz et al. 1999).

Changing the temporal scale over which an estimate of N_e is obtained can change the spatial scale. Schwartz et al. (1999) caution that historical estimates of N_{el} are likely to be biased upward because a population that is currently isolated might have experienced considerable gene flow during its past. Instead of estimating the N_e of the local population from which the samples were obtained, historical estimates may approach the global N_e for the species. Estimates of contemporary, local N_e will often be of most interest for understanding the current status of a population or the effects of a management strategy (Schwartz et al. 1999; Beaumont 2003a,b). However, comparing the contemporary N_e to estimates of historical levels provides an understanding of how recent environmental alterations have affected N_e (Crandall et al. 1999, Funk et al. 1999). Understanding whether a small \hat{N}_e was due to a past event or ongoing demographic processes is helpful in making manage-

ment decisions, so estimating N_e over different time scales should be considered with the caveat that spatial scales being examined with contemporary and historical estimates are likely to differ.

GENERAL CONSIDERATIONS RELATED TO ESTIMATION

Several assumptions are common to most estimation approaches (Table 1). Some of them can be addressed through sampling strategy; others are more problematic. I will discuss additional assumptions as they relate to specific estimators, as well as cases where the assumptions do not apply or have been relaxed.

With the major exception of the coalescent-based methods (which can use maternally inherited mtDNA sequences), most of the methods also assume that the genetic markers are biparentally inherited; I focus on such markers. The 2 primary types of biparentally inherited markers used to study wildlife populations are allozymes (Leberg 1996) and microsatellites (Bruford et al. 1996, Schwartz et al. 1998). The greater power resulting from use of highly polymorphic markers, as well as the assumption of selective neutrality (Table 1), make microsatellite loci more appropriate than allozymes; however, allozymes might be useful if they are sufficiently polymorphic and there is no obvious evidence of selection influencing allele frequencies. The high mutation rates of microsatellites also means that estimates of historic N_e will more strongly reflect recent history than estimates based on more slowly evolving markers. Newly developed types of biparentally inherited markers, such as single nucleotide polymorphisms (SNPs; Brumfield et al. 2003), should perform well with most estimators of N_{eV} ; however, they may produce more biased estimates of historical N_{el} than would be obtained with microsatellites (Morin et al. 2004). I will discuss applications using other types of markers in association with specific estimation approaches.

The precision of most estimators of N_e improves with the number of independent loci. Increasing the number of alleles examined can also improve precision. Typical recommendations are in the range of 10–20 highly polymorphic microsatellite loci; greater numbers of allozymes or SNPs would be necessary to obtain similar resolution. Most recommended sample sizes are ≥ 50 individuals; smaller sample sizes are necessary as N_e decreases. In very small populations, sample sizes might need to approach the N_e ; this could best be accomplished with noninvasive techniques for DNA col-

Table 1. Assumptions common to most approaches using genetic markers to estimate effective size (N_e).

Assumption	Comments
Population is sampled at random	
Genetic markers are selectively neutral and they should not be linked to markers under selection	Should be met by most markers used in genetic surveys. This assumption is probably better satisfied for microsatellites than for allozymes.
No subdivision of population	Sampling should be conducted to avoid combining genetically differentiated subpopulations. This may be a difficult assumption to meet.
No immigration	Relaxed in temporal method of Wang and Whitlock (2003), and several coalescent estimates of N_{eH} identify in state, and in estimates of Neighborhood size.
No overlapping generations	Relaxed in the temporal method of Jorde and Ryman (1995) or when several generations occur between temporal samples.
Stable population size	Relaxed in recently developed estimators for the temporal method and the coalescent method.
No mutation (for contemporary N_e) or the mutation rate and model is known (for historical N_e)	Assumption of no mutation is probably met for most studies examining contemporary N_e

Assumption of known rates and models of mutation is more difficult to address.

lection. In general, increasing the number of loci sampled will increase the precision of estimates to a greater extent than increasing the number of individuals sampled, but most studies would benefit from increasing both quantities.

It is difficult to discuss estimators of N_e without reference to the processes used to make inference about the parameter. Estimation procedures that equate \hat{N}_e to its expected value are moment estimators. Most moment estimators of N_e are based on expectations from classical population-genetics theory. These estimators can often be expressed in relatively simple equations and perform well under many situations. Until recently, most genetic methods for estimating N_e were moment estimators. Maximum likelihood (ML) estimators maximize the probability of the data for a given set of parameter values. Inference is made based on the likelihood function, and more information about allele frequencies is utilized than with the moment estimators (Wang 2001). Relative to some moment estimators, ML estimators tend to be less biased and more precise (Wang 2001, Berthier et al. 2002). Very recently, Bayesian estimation has become more widely used with the development of more efficient computational approaches (Beaumont and Rannala 2004). Bayesian estimators have the advantage of allowing incorporation of prior knowledge about the population and genetic markers, such as population size and variation in mutation rates, into the estimation process. Comparisons of the performance of the different types of estimators on data sets from natural populations often produce contradictory results with

regard to estimate bias and precision (see below), suggesting the relative merits of the approaches are not consistent across data sets. Since it is difficult to argue that 1 approach always performs best, it might be prudent to estimate N_e using >1 method.

ESTIMATING VARIANCE EFFECTIVE SIZE, N_{EV}

Temporal Methods

Moment Estimators.—Most estimates of N_{EV} are made using multiple samples from a population, with the samples separated by several generations. Changes in allele frequencies between samples, which reflect drift, are used to calculate \hat{N}_{EV} . A review and refinements of the approach are provided by Waples (1989).

To use this approach, samples are needed from at least 2 points in time, t_1 and t_2 . A commonly used formulation estimates the change in allele frequencies as:

$$\hat{F} = \frac{1}{K} \sum_{i=1} \frac{(x_i - y_i)^2}{(x_i + y_i) / 2 - x_i y_i}$$

where K is the number of alleles, and x_i and y_i are the frequencies of allele i at t_1 and t_2 , respectively (Nei and Tajima 1981). The idea is to measure genetic drift by determining the differences in allele frequencies between samples. The temporal allelic variance is affected by actual changes in allele frequencies and by sampling error. Thus, it is impor-

Table 2. Characteristics of genetic marker-based estimators of N_{eV} available to wildlife biologists.

Type of estimator	Type of inference	Number of sampling events	Scale of estimation period ^a	Citations/computer program ^b	Assumptions relaxed with approach
Temporal method	Moment	2	Contemporary	Waples (1989)	Nonoverlapping generations
	Maximum likelihood (ML)	2	Contemporary	Jorde and Ryman (1995) Anderson et al. (2000)/ MCLEEPS Laval et al. (2003) Wang (2001) and Wang and Whitlock (2003)/ MLNE	
	Bayesian	2 >2	Contemporary Historical and contemporary	Laval et al. (2003) Berither et al. (2002) and Beaumont (2003)/TMVP Tallmon et al. 2004/SUMSTAT	No Gene flow Stable population size
Gametic disequilibrium	Moment	1	Contemporary	Bartley et al. (1992)	
Heterozygote excess	Moment	1	Contemporary	Pudovkin et al. (1996)	
Rejection algorithm	Model-based	1	Contemporary	Ramakrishnan et al. (2004)	
Differences among populations	ML	1 ^c	Contemporary-historical	O’Ryan et al. (1998)/ DRIFTLIK	

^a This distinction is relative. Although temporal methods are typically thought of as measures of contemporary N_e , if the samples are taken many generations apart, the estimate of N_{eV} may be influenced by events long before the present time. Bayesian methods based on >2 temporal samples have the potential to look at changes in N_{eV} over time.

^b When several citations are available, a representative citation reviewing the approach is provided. Computer programs to assist in analysis are listed when known.

^c Although only 1 temporal sample is required, samples are required from 2 populations that have been isolated for a known number of generations.

tant that sample sizes be factored into any \hat{N}_e based on this method. When sampling without replacement (Waples 1989), it is possible to estimate N_e using:

$$\hat{N}_{ev} = \frac{t}{2 \hat{F} - \left[\frac{1}{2S_0} - \frac{1}{2S_t} \right]}$$

where S_0 and S_t are the sample sizes used to obtain the allele frequencies from the first and second samples, and t is the number of generations between the samples. Waples (1989) also discusses a variation on this approach to be used when the genetic samples are obtained with replacement. Both these sampling schemes will potentially be used in wildlife studies since samples from game populations are often collected through harvests (without replacement), while samples from threatened or rare species are often obtained using non-destructive approaches (with replacement).

Maximum Likelihood and Bayesian Estimators.—Williamson and Slatkin (1999) presented a ML approach that produced less biased and more precise of N_{eV} than those obtained with the moment estimator. This method required considerable computing time and was not suitable for highly

polymorphic markers. More computationally efficient ML and Bayesian methods suitable for microsatellites are now available (Table 2). Tallmon et al. (2004) provide a comparison of some of these estimators.

Sampling Considerations.—Precision and accuracy of \hat{N}_eV can be improved by increasing the number of loci examined, the number of alleles examined, the sample size, or the amount of time between temporal samples (Waples 1989, Wang 2001, Berthier et al. 2002). Increasing the amount of time between temporal samples is the most difficult, and so it is rarely considered. However, for these methods to estimate N_{eV} , drift has to occur; little drift will occur if N_{eV} is large unless the number of generations between samples is large. Thus, these methods tend to be most suitable when $N_{eV} < 100$. Sampling more than twice in a temporal series also improves the precision of \hat{N}_{eV} .

The long generation times of many wildlife species will make it difficult to obtain temporal samples >1 generation apart; in some cases, even that amount of time will require studies lasting many years. Obtaining samples separated by several generations is especially important when generations overlap (Jorde and Ryman 1995). One potential so-

lution to this problem is to compare the genetic characteristics of contemporary populations to those of museum specimens. This approach has been used to obtain \hat{N}_{eV} for grizzly bears (*Ursus arctos*), greater prairie-chickens (*Tympanuchus cupido*), and Northern leopard Frogs (*Rana pipiens*; Miller and Waits 2003, Johnson et al. 2004, Hoffman et al. 2004, respectively). Use of this approach will be limited to populations for which there are relatively large collections of museum specimens.

When used in conjunction with allozyme loci, the moment estimator overestimated effective size of very small populations (Richards and Leberg 1996). This bias was reduced when F and \hat{N}_{eV} were based on highly polymorphic microsatellite loci (Luikart et al. 1999, Spencer et al. 2001). The moment estimator has the potential to be biased when it is based on loci where only 1 allele is common and alternate forms are rare (Waples 1989), and it does not perform as well as ML approaches in such cases (Berthier et al. 2002). Scribner et al. (1997) and Jorde et al. (1999) discuss use of temporal method for dominant genetic markers where it is difficult to identify heterozygotes.

Assumptions.—In addition to the typical assumptions (Table 1), potential for errors in genotyping to influence differences in allele frequencies is of particular concern when using the temporal method. Scribner et al. (1997) and Miller and Waits (2003) discuss this problem with regard to minisatellites and microsatellites. Because genotyping errors would increase the temporal variation in allele frequencies, they lead to underestimates of N_{eV} . Regardless of the marker used, investigators should take steps to reduce genotyping error; several strategies for reducing genotyping errors have been suggested for microsatellites (Taberlet et al. 1999, Miller and Waits 2003, Mckelvey and Schwartz 2004).

Many temporal approaches assume that N_e is relatively stable during the period between samples (Table 1; Waples 1989). If N_e is changing, the estimate obtained is that of the harmonic mean of N_e in the intervening generations. The harmonic mean differs from the more arithmetic mean, in that it is weighted toward the smallest values of N_e that occurred between the sampling interval. It is not surprising that the generations with the smallest N_e would have a large effect on genetic drift; 1 severe bottleneck between 2 samples is likely to have a much greater effect on temporal variance in allele frequencies than several generations where N_e was large.

Variation in N_e is expected to occur during the collection of temporally spaced samples. Two estimators now allow for variation in the population

size between temporal samples (Table 2). They assume that the population be sampled >2 times and that the population is increasing or decreasing exponentially during the sampling period. These approaches provide an estimate of how N_{eV} has changed over time.

Violation of the assumption of nonoverlapping generations is unlikely to have a large effect on \hat{N}_{eV} as long as demographic parameters of the population are stable and time period between samples is long (Waples 1989, Jorde and Ryman 1995). If only a few generations have occurred between samples, less biased results could be obtained following the approach of Jorde and Ryman (1995). Modifying the temporal method for overlapping generations requires rough estimates of age-specific reproduction and survival (Jorde and Ryman 1995, Turner et al. 2002). These methods assume that N_{eV} , the demographic rates, and age structure are stable between the temporal samples.

Violation of the assumption of no migration can have complex effects on \hat{N}_{eV} (Fig. 2; also see Waples 1990). If populations are in equilibrium with surrounding populations with regard to the effects of gene flow on allele frequencies, migration results in an overestimate of N_e unless the number of generations between samples is short (Fig. 2, solid line). This bias is greatest if the samples are collected many generations apart. If gene flow is great, the estimate obtained would more closely reflect the size of all the populations exchanging migrants than the N_e of the sampled population. Alternately, if populations are not in equilibrium with regard to gene flow (Fig. 2, dotted line), N_e can be dramatically underestimated by the temporal method. This occurs because recent migration events, such as translocations near the population of interest, can cause gene frequencies to change rapidly. Investigators applying temporal methods tend to minimize the effects of immigration in the discussion of their results. Given that migration can cause an overestimate or an underestimate of N_e , estimates for populations experiencing gene flow should be treated with great caution.

Recently, Wang and Whitlock (2003) presented a ML estimator for populations experiencing gene flow. This method estimates migration rates, as well as N_e for study populations. The method assumes that all sources of migrant individuals were identified and were characterized with genetic markers. It also assumes that the first sampling event did not change the pool of reproductive individuals. This can be accomplished through nondestructive sampling, sampling after reproduction, or sampling

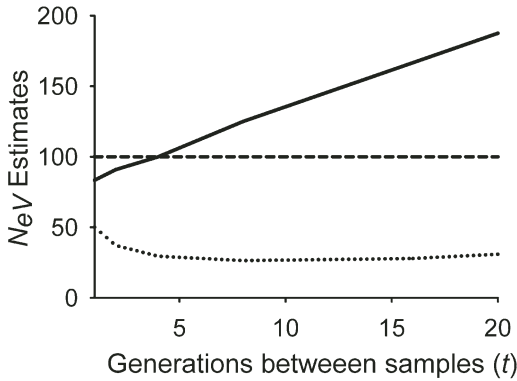


Fig. 2. Influence of migration and the number of generations between samples on estimates of N_{eV} when populations are in equilibrium (solid line) and not in equilibrium (dotted line) with surrounding populations with regard to the effects of gene flow on allele frequencies. The dashed line represents the effective size of the hypothetical population; estimates above and below it are biased (modified from Wang and Whitlock 2003).

from a population that is much larger than the number of individuals sampled (Wang and Whitlock 2003). It is important to realize that an \hat{N}_{eV} obtained with this approach no longer reflects the drift that is occurring in the sampled population. Rather, \hat{N}_{eV} reflects what drift would be if migration was terminated. Thus a small \hat{N}_{eV} might not be of concern as long as gene flow was maintained.

Examples.—An application of moment and ML estimators is a study of 4 semi-isolated populations of greater prairie-chickens. To obtain a second temporal sample, Johnson et al. (2004) obtained the genotypes of 125 museum specimens. Approximately 30 generations separated the samples. Estimates of \hat{N}_{eV} based on the assumption of no migration among the subpopulations were many times larger than the estimates (15–22 birds per subpopulation) obtained with Wang and Whitlock's (2003) approach allowing migration. This result indicates the importance of using an estimator that allows for migration when the population is not closed to immigration. From a management perspective, these findings indicate that these semi-isolated populations are heavily dependent on immigration and that loss of connectiveness would result in rapid erosion of genetic diversity in each of the population fragments.

A comparison of different estimators of N_{eV} for populations of feral cats (*Felis catus*) found the Moment estimator and a demographic model produced more realistic and less variable estimates than the Bayesian approach (Kaeuffer et al. 2004). In studies of grizzly bears and natterjack toads (*Bufo calamita*) estimates from ML estimators and the mo-

ment estimator were quite similar (Miller and Waits 2003, Rowe and Beebe 2004). Hoffman et al. (2004) found that Wang and Whitlock's estimator did not perform well when the genetic composition of source populations of leopard frogs that immigrated to their study populations was poorly known. Additional comparison of estimators across a range of conditions is needed to obtain a better sense of their relative performance. Other applications of the temporal method of interest to wildlife biologists include Funk et al. (1999), Jehle et al. (2001), and Lucchini et al. (2004).

Linkage Disequilibrium

Linkage disequilibrium is the lack of independence of the occurrence of alleles at different loci. In a large randomly mating population, there should be no correlation between the presence of alleles at 2 neutral loci, unless the loci were linked physically by their proximity on the same chromosome (Bartley et al. 1992; Table 2). However, linkage disequilibrium can occur in small populations through random drift, making it possible to estimate N_{eV} (Hill 1981, Bartley et al. 1992). Cases where there is little disequilibrium, coupled with a small sample size, can result in a negative \hat{N}_{eV} . In this case, N_{eV} is typically considered to be infinitely large (Bartley et al. 1992).

The primary advantage of this moment estimator over the temporal method is that it requires only 1 sample. However, inadequate sample sizes and numbers of loci can result in imprecise estimates (Bartley et al. 1992).

Because the method assumes that the population has discrete generations, it is sometimes applied to a single cohort of individuals. In this case, rather than \hat{N}_{eV} , the method estimates the effective number of breeding individuals, N_{eb} (Schwartz et al. 1998). Note that the temporal method can also be used to estimate N_{eb} by examining the allele frequency differences between a cohort of offspring and the adult population. Estimates of N_{eb} might be useful for comparing populations, but it is difficult to use them to estimate rates of loss of genetic variation for populations with overlapping generations. This approach has not been widely applied to populations of terrestrial vertebrates.

Heterozygote Excess

In very small populations, there will be chance genotype differences between the sexes. Because of the small chance-differences in allele frequencies between males and females, their offspring will actually be more heterozygous than would be expected based on the overall allele frequencies in

Table 3. Characteristics of genetic marker-based estimators of historical^a N_{eb} available to wildlife biologists.

Type of inference	Citations/computer program ^b	Nuclear molecular markers	Assumptions relaxed with approach
Moment	Nei (1987)	Allozymes and microsatellites	
ML	Nielsen (1997)/MISAT, Xu and Fu (2004) Nielsen (2000)	Microsatellites SNPs	
	Beerli and Felsenstein (1999, 2001)/MIGRATE	Allozymes, microsatellites	No migration
	Polanski and Kimumel (2003)	SNPs	Stable population size
Bayesian	Nichols and Freeman (2004)/BAYALLELE	Microsatellites	No variation in mutation rate, no migration
	Wilson et al. (2003), Beaumont et al. (2002) Beaumont (1999)/MSVAR	Sex-linked microsatellites Microsatellites	Stable population size Stable population size

^a Although generally thought to represent N_e over many generations, they may be strongly influenced by recent events. Estimates based on markers with high mutation rates, such as microsatellites, are more likely to reflect recent effective sizes of the populations than markers with low mutation rates.

^b When several citations are available, a representative citation reviewing the approach is provided. Computer programs to assist in analysis are provided when known.

the population. Pudovkin et al. (1996) used this observation to develop a moment estimator for N_{eb} (Table 2). In this case, N_{eb} is the effective number of individuals that produced the cohort being sampled. Balloux (2004) provides corrections for biases associated with Pudovkin et al.'s (1996) estimator.

This approach has many of the same benefits and drawbacks as the linkage disequilibrium method. An additional concern is the assumption of random mating (Pudovkin et al. 1996, Schwartz et al. 1998), which would limit the approach's value in wildlife populations with social structure. Using simulations, Luikart and Cornuet (1999) found no large bias in estimates resulting from polygamous, polygynous, and monogamous mating systems when they used an unbiased estimator of H_{HW} . However, for the same number of loci and individuals, the confidence intervals for \hat{N}_{eb} are much larger for monogamous than polygamous populations.

Low precision is another drawback to this approach. Even with a very large sample size and number of alleles, it is unlikely that reasonable confidence intervals will be obtained if $N_{eb} > 10$ (Luikart and Cornuet 1999). In applying the method to natural populations, \hat{N}_{eb} were often infinitely large because N_{eb} was large or because insufficient numbers of offspring or polymorphic loci were examined. The requirement of a large sample size from a population with very small N_{eb} will limit the usefulness of this approach for wildlife species. Furthermore, wildlife populations frequently exhibit heterozygote deficiencies due to a variety of factors including internal subdivision, null alleles, and allelic dropout (Oosterhout et al. 2004); these factors

could bias \hat{N}_{eb} . One potential use for the approach is to estimate N_{eb} for population subdivisions by sampling juveniles prior to dispersal; however, this would require knowledge of subdivision boundaries (Balloux 2004).

Rejection Algorithm for Estimating Sex-specific N_e

If an exhaustive sampling of progeny and their associated mothers (or fathers) is possible, Ramakrishnan et al. (2004) provide a method for estimating the effective size of the parents of the unsampled sex. The approach uses a rejection algorithm that compares 3 statistics to expectations based on simulation modeling. This method will be most useful when 1 sex is very difficult to sample and the other is not. For example, they estimated the N_e of breeding males, N_{ebm} , for a population of fruit bats (*Cynopterus sphinx*) from which they had samples of mothers and their offspring. This method was least biased when N_{ebm} was much smaller than the number of males (Ramakrishnan et al. 2004), a condition that is most likely in polygamous species.

Comparing Recently Isolated Populations

Some situations lend themselves to estimation of N_{eV} over a longer time period. If 2 or more populations were known to be joined together at some known earlier time, then became isolated, it is possible to estimate N_{eV} by assuming that that time since separation is sufficiently short so that mutations are unlikely (O'Ryan et al. 1998; Table 2). This approach would be particularly useful for isolated populations established through translocations or recent habitat

Table 4. Characteristics of genetic, marker-based estimators of N_e available to wildlife biologists.

Type of N_e	Scale of estimation period ^a	Type of inference	Citation/computer program	Properties
Neighborhood size	Historical	Moment	Rousset (2000), Leblois et al. 2003)	Useful in populations with continuous spatial distributions
Identity in state	Historical	Moment	Vitalis and Couvet (2001a,b) /ESTIM	Allows for migration
Changes in recently established populations	Contemporary-dependent on the amount of time since population establishment	Bayesian	Estoup et al. (2001) Estoup and Clegg (2003)	Allows for changes in population size

^a This distinction is relative. For example, while Rousset's (2000) estimator of neighborhood size reflects historical N_e , it is most strongly influenced by events occurring in the last 10 generations. Computer programs to assist in analysis are provided when known.

fragmentation. Rowe and Beebee (2004) used this method to obtain \hat{N}_{eV} for 2 populations of toads; one of which served as a source of individuals founding the second population. The degree to which this measure estimates contemporary or historical N_{eV} depends on the amount of time the populations were isolated. If a bottleneck occurred early in the isolation event after which the population expanded, the estimate will be more strongly affected by that population size than by the N_e of more recent generations.

ESTIMATING INBREEDING EFFECTIVE SIZE, N_{EI}

Moment Estimators

Classical population genetics provides moment estimators for N_{eI} based on the equilibrium relationship between mutation rate (u), H_{HW} , and N_e . This relationship is affected by the mutation model that best applies to the genetic markers used in the assay. Based on expectations under the stepwise-mutation model (SMM, Nei 1987), it is possible to estimate N_{eI} from microsatellite data using:

$$\hat{N}_{eI} = \left[\frac{1}{(1 - H_{VW})} \right]^2 - 1$$

A similar estimate from allozymes, based on an infinite-alleles model (IAM; Nei 1987), would be:

$$\hat{N}_{eI} = \frac{H_{VW}}{4u(1 - H_{VW})}$$

Studies using this approach to estimate N_{eI} in wildlife populations include Paetkau et al. (1998), van Hooft et al. (2000), and Spong et al. (2000).

Assumptions.—In addition to the standard assumptions (Table 1), these models assume that the level of heterozygosity in a population is due only to mutation and drift and that these forces

are in equilibrium. This approach also assumes that the correct mutation model and the mutation rate are known and are the same across loci. Authors often use an average mutation-rate for a group of taxa. However, reported estimates of mutation probabilities for microsatellites and other markers can vary widely. Use of the wrong rate can have a large effect on estimates of long-term N_{eI} .

Choice of mutation model can also influence \hat{N}_{eI} , with higher estimates being obtained with the SMM. Although the SMM model is frequently used for microsatellites, the IAM model has been occasionally used, and there is considerable evidence that the mutation process is more complex than either of these models (Di Rienzo et al. 1994). The correct mutation model and mutation rate are probably influenced by characteristics of individual loci (Ellegren 2000). Rather than select 1 mutation rate and model, it would be more conservative to evaluate the effects of different appropriate mutational models and rates on \hat{N}_{eI} . Making incorrect assumptions about mutational models or rates is not a major problem if the goal is to compare \hat{N}_{eI} among populations assayed with the same markers and not to make inferences on the rate of accumulation of inbreeding (Lehman et al. 1998). However, in such cases, there is no real reason to estimate N_{eI} , as comparing estimates of H_{HW} provides the same information.

An assumption often not considered when applying these models is that the loci used to estimate H_{HW} are selected at random from the population of loci with the same mutation rate. Often, investigators screen genetic markers, and particularly microsatellites, for polymorphism before they are included in a study. If invariant loci were excluded from an analysis, but not from studies used to estimate the mutation rate, the mutation rate of the loci in the study may be higher than the value of u used in the estimator. This would lead to an underestimate of N_{eI} (van Hooft et al. 2000).

Coalescent Approaches

In recent years there have been several advances in the estimation N_{eI} , based on the use of coalescence theory (i.e., Nielsen 1997, Wakeley 2001). Coalescence uses relationships between genes, as influenced by mutation and genealogy, to understand evolutionary phenomena (Kingman 1982). Taking a sample of genotypes, coalescence methods trace backward in time to identify events that occurred since the individuals in the sample shared a common ancestor (Fu and Li 1999). Thus, they are based on genealogic relationships between individuals in the sample rather than on estimates of population parameters like H_{HW} . Application of this approach to estimate parameters has, perhaps, been the most active area in theoretical population genetics over the last decade (Fu and Li 1999).

The parameter estimated in most coalescence approaches is θ , which is defined as $4N_e u$ when based on biparentally inherited markers. To obtain \hat{N}_{eI} from $4N_e u$ it would be necessary to assume a mutation rate (u) for the type of genetic marker using estimates from the literature. The same concerns about use of inaccurate estimates of θ and inappropriate mutational models discussed for the moment estimators apply here. Alternately, estimates of θ from different populations can be compared directly under the relatively safe assumption that mutation rates will be similar (Neigel 1996).

Examples.—Several coalescent-based estimators have been developed (Table 3); these are often less biased but more computationally intensive than the moment estimators. One application of this approach was an attempt to estimate θ in a population of baboons (*Papio cynocephalus*) using Nielsen's (1997) approach (Storz et al. 2002). The population experienced a dramatic decline from approximately 2,500 baboons in 1964 to about 480 by the mid-1970s due to habitat changes. Historical \hat{N}_{eI} based on microsatellite loci, was 2.2–7.2 times higher than current N_e . Storz et al. (2002) used this observation to argue that the population had not yet become inbred to the extent predicted by its current size, and to argue that the current population size was far below historical norms. A large \hat{N}_{eI} was also reported for leopards (*Panthera pardus*) in Tanzania (Spong et al. 2000). Both studies used literature estimates of mutation rates to estimate N_{eI} from θ . These high estimates of N_{eI} may be somewhat biased (Spong et al. 2000, Storz et al. 2002) by deviations from the assumptions associated with Nielsen's (1997) estimator. For example, population subdivision may cause the N_{eI} to exceed N_e (Sugg et al. 1996). Additionally, the populations

of leopards or baboons were not closed to migration, which could cause overestimates of θ and N_{eI} .

Assumptions.—In general, most of the first generation of coalescent models make many of the same assumptions that were discussed for moment estimators of N_{eI} . There is also an assumption that the N_{eI} has been large for a long period of time. These approaches are not robust for populations with $N_{eI} < 50$, which may limit their usefulness in some populations of endangered or threatened species.

The assumption that populations are closed to immigration has been relaxed (Table 3), making it possible to obtain separate estimates of N_{eI} and m for multiple subpopulations (Beerli and Felsenstein 2001). The most robust estimates of N_{eI} will be obtained when genetic information from all sources of immigrants are included in the analysis, however, procedures exist to address immigration from unsampled sources (Beerli and Felsenstein 2001).

A Bayesian approach for simultaneously estimating N_e , u , and m for a set of populations has been recently described by Nichols and Freeman (2004). Using loci with high mutation rates, it is possible to distinguish the effects of mutation, migration, and drift on patterns of interpopulation differentiation (Table 3); efficiency is improved when the user can supply information on relative mutation rates and population sizes.

The assumption of population stability has been relaxed by recent advances in the estimation of N_{eI} aimed at detecting past population expansions or contractions (Table 3). Using a Bayesian approach, information on past population history can be used to estimate N_{eI} (Beaumont 1999). The method was applied to microsatellite data for the northern hairy-nosed wombat (*Lasiorhinus kreffthii*). The species underwent a bottleneck from thousands of individuals to just 20–30 individuals in 1981. Beaumont (1999) found that the population had been declining over a much longer period and more dramatically than the historical data implied. Using this approach, Luchini et al. (2004) found evidence for a long-term decline of a populations of wolves (*Canis lupus*).

ESTIMATING OTHER FORMS OF N_E Neighborhood Size

Estimates of N_e are made for populations; it is assumed the individuals sampled represent a random sample of all the members of a population with no internal spatial structure. However, this concept of population makes little sense for species with large, continuous distributions. With no major discontinuities in the distribution, it would be difficult to

determine the extent of the area occupied by a population. When there are no identifiable populations from which to sample, it is hard to apply estimation approaches that include a measure of immigration from other surveyed populations.

For species with relatively continuous distributions, Wright (1943) proposed the concept of neighborhood size. Genetic differentiation is predicted to increase with distance; this is referred to as isolation by distance (IBD). Under IBD, neighborhood size is estimated as $4\pi D\sigma^2$ where D is the population density of adults, and σ^2 is the average squared dispersal distance. This formulation of neighborhood size is equivalent to N_{el} (Hedrick 2000) and depends on most of the standard assumptions (Table 1). Density affects loss of genetic diversity, as would N_{ef} ; however, the relationship is not direct because of the influence of gene flow, which is reflected by the measure of dispersal. Neighborhood size has been estimated demographic data (e.g. Wasser and Elliot 1991), but until recently, there have been few methods of estimation based on genetic markers (Table 4).

A moment estimator of $D\sigma^2$, the variable portion of $4\pi D\sigma^2$, was developed by Rousset (2000) and applied to a population of Banner-tailed kangaroo rats (*Dipodomys spectabilis*). This estimator is based on multilocus genetic similarity and the spatial distance between pairs of sampled individuals. The method performs well under a variety of distributions of dispersal distances. The estimator of $D\sigma^2$ will be most precise and least biased for loci with levels of polymorphism comparable to microsatellites (Leblois et al. 2003). Although the estimator has the potential to be affected by historical events, Leblois et al. (2004) found estimates of $D\sigma^2$ reflected recent density. Population reductions and expansions much more than 10 generations in the past had only minor effects on $D\sigma^2$.

Sampling at too large or small a spatial scale is likely to bias estimates of $D\sigma^2$ (Leblois et al. 2003). The recommended scale of sampling is an area of $10\sigma * 10\sigma$. Thus some knowledge of average dispersal distances of offspring from their parents' home ranges is helpful. The estimate of σ does not have to be precise; sample areas differing by a factor of 2 from the recommended area, still yielded robust estimates of $D\sigma^2$. Obviously, sampling areas might be quite large for highly mobile species. Sampling a small area of high density might bias estimates; ideally the area sampled should be centered in an area of similar density that is >4x the size of the sampled area (Leblois et al. 2004). While complete sampling of individuals in this area would be

ideal, Leblois et al. (2003) indicated that samples of approximately 100 individuals should be sufficient to estimate $D\sigma^2$. Although this method would be applicable to many wildlife populations, spatial sampling will need to be much more systematic than is typical in wildlife genetic studies.

Identity in State

An interesting approach that has yet to be widely applied is the use of single-locus and 2-locus probabilities of identity in state (IIS) to provide a moment estimate of N_e in the presence of migration (Vitalis and Couvet 2001a). The probability of IIS for a single locus is the probability that the 2 alleles found in a sampled individual will be identical. Other single-locus measures that are calculated include the probability that 2 randomly sampled alleles from a subpopulation, or from the pool of immigrants, are identical. Similar measures of IIS are calculated for alleles at independent loci, reflecting inter-locus disequilibria that can occur in small populations (Vitalis and Couvet 2001a). Samples should be obtained from the set of populations thought to be exchanging genetic material. Parameters estimated in this analysis include N_{eIIS} and per-generation immigration rate (m) for each subpopulation in the analyses.

The model estimates the size of an ideal population that would have the same 2 locus-genetic structure as the actual population. This measure of N_e was not directly compared to N_{eV} and N_{el} (Vitalis and Couvet 2001a), but it is expected that these measures would converge for populations at equilibrium for mutation and drift in the absence of gene flow.

Least biased \hat{N}_{IIS} are obtained when N_e is small. However, when 8–12 unlinked loci were examined, reasonable \hat{N}_{IIS} were obtained for populations where $N_e = 100$ (Vitalis and Couvet 2001a). To estimate effective sizes for larger populations, more tightly linked loci will be necessary (Vitalis and Couvet 2001b). This method relaxes the assumption that the population is closed to immigration (Table 4). It assumes that the populations are at equilibrium between mutation, drift, and migration; populations that are declining, increasing, or experiencing changing levels of gene flow due to fragmentation or translocations will violate this assumption. Simulations indicate that violation of this assumption biased estimates of m more than \hat{N}_{IIS} (Vitalis and Couvet 2001a).

Changes in Recently Established Populations

An \hat{N}_e for a recently established population can be obtained by comparing several different genetic variables (heterozygosity, allele frequencies, and

the distribution of sizes of microsatellite alleles) from the population to the population from which it was founded (Estoup et al. 2001; Table 4). Several components of genetic change are measured because they respond differently to the established population's demographic history. In this case, N_e is the size of an ideal population experiencing the same genetic drift, loss of alleles, and inbreeding as the actual population. This approach is difficult to compare directly to N_{ef} and N_{eV} , and N_e is estimated for different portions of the population's history. This Bayesian approach allows incorporation of information on the population's history into the estimation process (Estoup et al. 2001).

Using data from *Bufo marinus*, a toad introduced to several Pacific islands, Estoup et al. (2001) made estimates of N_e for the populations colonizing the islands, as well as for the N_e after the population stabilized following a period of rapid population growth and decline. They had less success estimating population parameters during this boom/bust period, perhaps because this transition period was short and the power to detect rapid changes in population was reduced by losses of genetic variation during the establishment of the populations. An encouraging feature of this approach is that it allowed estimation of N_e in populations during the last 150 years. While this may not represent an estimate of contemporary effective size, it does cover a time period relevant to wildlife biologists.

Such an approach might be applicable to many situations where there is considerable information about the history of populations, such as those under intensive management or those monitored following reintroduction. There is no assumption that the populations are in equilibrium with regard to drift, mutation, and gene flow, but use of this technique to estimate N_e and other population parameters in a nonequilibrium system will require considerable genetic information (Estoup et al. 2001). Using this approach, Estoup and Clegg (2003) obtained estimates for the number of founders and the average N_e for populations of a songbird (*Zosterops lateralis*) that colonized several Pacific Islands over the last century. Because of computational limitations, this approach is most suitable to studying isolated populations that experienced a founder event or other bottleneck (Estoup and Clegg 2003).

MANAGEMENT IMPLICATIONS

Estimating effective population size can provide insight into how fast a population is losing genetic variation and perhaps its vulnerability to extinction (Schwartz et al. 1998). With the great diversity of

available estimators, it is important for the wildlife biologist to identify the best approach to use. There is an unfortunate lack of comparative studies that evaluate the relative merits of different approaches (see Tallmon et al. 2004 for a notable exception). Without independent evaluations of different approaches using simulations and data, it is difficult to predict which approaches will stand the test of time. While valuable, comparative studies of experimental and natural populations will be difficult to interpret because it is difficult to control all of the parameters affecting N_e or to know the true value of N_e for any population.

The importance of the temporal method is demonstrated by the level of activity associated with the development of new estimators. Choice of an estimator should be based on whether the population is rapidly changing size or open to migration (Table 2). When multiple temporal samples are available along with information on temporal variation in N_e , a Bayesian approach might be the most appropriate. The biggest merit of heterozygote excess and linkage disequilibrium methods is the requirement of only 1 sample. Neither of these single-sample approaches have been widely used because they have restrictive assumptions and low precision. Given the importance of contemporary N_e for conservation planning, steps should be taken to make temporal sampling more practical. In particular, samples of populations of management interest should be collected and stored in preparation for an analysis of temporal variation in allele frequencies at some future date.

For estimates of historic N_e the coalescent-based approaches hold the most promise. The choice of estimator will depend on whether the population is open to migration or changing in size (Table 3). Care should be taken in applying these methods to populations with continuous distributions; estimation of neighborhood size should be considered in such cases. Although estimates of historic N_e are often equated with N_e over evolutionary time, neighborhood size and coalescent methods applied to recently fragmented or bottlenecked populations are examining the accumulation of inbreeding over time scales of relevance to managers. The potential of estimators of Vitalis and Couvet (2001a) and Estoup et al. (2001) deserve further evaluation.

The effects of violation of assumptions that do not hold for most wildlife populations are still unclear. Although approaches exist to address failure of assumptions related to closure and population stability, less progress has been made in relaxing the assumption of nonoverlapping generations.

Ignoring or failing to detect metapopulation and social structure is also likely to have an effect on N_{ef} and N_{eV} (Chesser et al. 1993, Nunney 1999).

Estimates of N_e based on genetic markers nicely compliment information obtained from demographic approaches (e.g. Nunney and Elam 1994). If the temporal method produces a different estimate of N_{eV} , it suggests that either the demographic model missed important factors affecting the effective size of the population (e.g., temporal variation in size; Frankham 1995b) or that the genetic approach had an unaddressed bias. Identification of the cause of the discrepancy will provide new information on the factors affecting loss of genetic diversity in a population.

While there are still difficulties in applying genetic estimators of N_e to wildlife populations, the options and opportunities to obtain information on this important parameter have greatly expanded over the last decade. It has now become practical to obtain estimates of both contemporary and historical population size. Using proper caution with regard to potential biases associated with the violation of assumptions, there is little reason why information on N_e can not be obtained from most populations and be used to guide management decisions.

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