Distortion of Allele Frequency Distributions Provides a Test for Recent Population Bottlenecks

G. Luikart, F. W. Allendorf, J.-M. Cornuet, and W. B. Sherwin

We use population genetics theory and computer simulations to demonstrate that population bottlenecks cause a characteristic mode-shift distortion in the distribution of allele frequencies at selectively neutral loci. Bottlenecks cause alleles at low frequency (<0.1) to become less abundant than alleles in one or more intermediate allele frequency class (e.g., 0.1–0.2). This distortion is transient and likely to be detectable for only a few dozen generations. Consequently only recent bottlenecks are likely to be detected by tests for distortions in distributions of allele frequencies. We illustrate and evaluate a qualitative graphical method for detecting a bottleneck-induced distortion of allele frequency distributions. The simple novel method requires no information on historical population sizes or levels of genetic variation; it requires only samples of 5 to 20 polymorphic loci and approximately 30 individuals. The graphical method often differentiates between empirical datasets from bottlenecked and nonbottlenecked natural populations. Computer simulations show that the graphical method is likely ($P > .80$) to detect an allele frequency distortion after a bottleneck of $\leq$20 breeding individuals when 8 to 10 polymorphic microsatellite loci are analyzed.

Identifying populations that have experienced a severe reduction in size (i.e., a bottleneck) is important because bottlenecks can increase demographic stochasticity, rates of inbreeding, loss of genetic variation, and fixation of mildly deleterious alleles, thereby reducing evolutionary potential and increasing the probability of population extinction (Brakefield and Saccheri 1994; Frankel and Soule 1981; Frankham 1995a,c; Hedrick and Miller 1992; Jimenez et al. 1994; Lande 1988, 1994; Mills and Smouse 1994; Newman 1996; Ralls et al. 1988; Vrijenhoek 1994; but see Bryant et al. 1986; Goodnight 1987).

It is especially important to identify *recently* bottlenecked populations (i.e., populations bottlenecked within the past few dozen generations), because such populations may not yet have had time to adapt to the problems often caused by small population size and therefore may have a high risk of extinction. The more recent a bottleneck, the greater the probability that the deleterious effects of a bottleneck can be avoided or minimized by mitigative management procedures, such as habitat enhancement or introduction of immigrants. Recently bottlenecked populations are likely to have lost rare alleles, but may still contain substantial heterozygosity and quantitative genetic variation which are lost more slowly than allelic variation, and which influence fitness in current environments more than allelic variation (Allendorf 1986; Denniston 1978; Lande and Barrowclaugh 1987; Leberg 1992; Nei et al. 1975). Therefore, if biologists recognize that a population has been recently bottlenecked, they may be able to minimize loss of heterozygosity and quantitative genetic variation.

Identifying recently bottlenecked populations may also allow biologists to minimize or reverse the reduction of fitness and the fixation of deleterious alleles that often result from bottlenecks (Backus et al. 1995; Hedrick 1995; Newman 1996; Spielman and Frankham 1992). Unfortunately it is often difficult to identify recently bottlenecked populations because historical population sizes and levels of genetic variation are seldom known.

Our objective is to use empirical data and computer simulations to illustrate and evaluate a qualitative graphical method for identifying populations that have recently been bottlenecked. The graphical method requires no data on historical population sizes or historical levels of genetic variation; it requires only measurements of allele frequencies from 5 to 20 polymorphic loci in a sample of approximately 30 individuals. The method involves comparing the distribution of allele frequencies observed in a population suspected to have been bottlenecked to the distribution expected in a nonbottlenecked population.

We define a "nonbottlenecked" population as one that is thought to not have been recently bottlenecked and is there-

From the Division of Biological Sciences, University of Montana, Missoula, MT 59812 (Luikart and Allendorf), the Laboratoire de Modelisation et de Biologie Evolutive, I.N.R.A./U.R.L.B., Montpellier, France (Cornuet), and the School of Biological Science, University of New South Wales, Sydney, Australia (Sherwin). G. Luikart is currently at the Laboratoire de Biologie des Populations d'Altitude, CNRS, Université Joseph Fourier, BP 53 F-38041, Genoble Cedex 9, France. We thank S. Forbes, D. Tallmon, and A. Taylor for comments on earlier versions of this manuscript. P. England provided helpful discussions and comments. Funding for analyses of mountain sheep samples was provided by the Boone and Crocked Club and the National Rifle Association. J. Hogg and S. Forbes kindly provided unpublished data. Mountain sheep tissue samples were provided by J. McCarthy, J. Cross, and J. Firebaugh (Montana Department of Fish Wildlife and Parks), O. Dyre, H. Langin, A. Peat, A. Wolterson, and B. Lincoln (British Columbia Ministry of Environment, Wildlife Branch), Jon Jorgenson (Alberta Fish and Wildlife Service), M. Miller (Colorado Department of Fish and Wildlife), J. Hogg, and Marco Festa-Bianchet. Address correspondence to Gordon Luikart at the address above or e-mail: gluikart@ujf-grenoble.fr.

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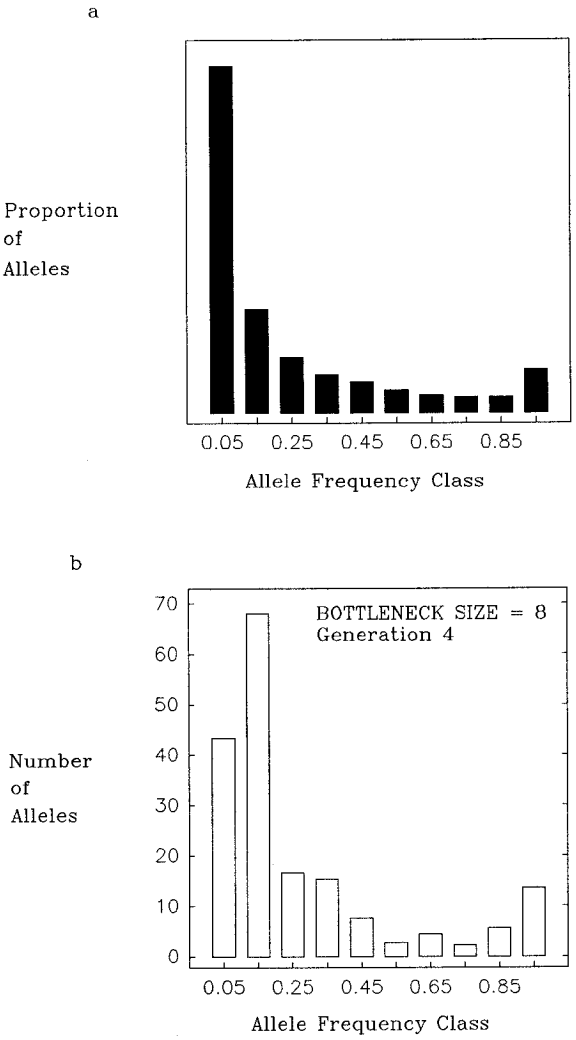

Figure 1. **(a)** Distribution of allele frequencies expected for loci evolving under the infinite allele model of mutation (IAM) in a nonbottlenecked population at mutation-drift equilibrium (Nei et al. 1976). Black bars represent the proportion of alleles expected in each of 10 allele frequency classes. The mean heterozygosity expected for a random sample of loci having the illustrated distribution is 0.40. **(b)** Distribution of allele frequencies expected (for a sample of 50 loci) in a population bottlenecked to eight breeding individuals (N_e = 8) for four generations. Open bars represent the number of alleles expected in each of 10 allele frequency classes. Expected numbers of alleles were calculated as the mean of 500 replicate bottleneck simulations.

fore likely to be near mutation-drift equilibrium. For selectively neutral loci, allele number and frequency distribution in a natural population results from a dynamic equilibrium between mutation and genetic drift. This "mutation-drift" equilibrium will be approximately reached if the effective population size (N_e) remains stationary for (4–10 multiplied by N_e) generations (Nei and Li 1976).

The expected distribution of allele frequencies for neutral loci in a nonbottlenecked population has been established. Nonbottlenecked populations that are near mutation-drift equilibrium for selectively neutral loci are expected to have a large proportion of alleles at low frequency (Figure 1a). The expected proportion of alleles at low and intermediate frequencies will vary with the mutation rate and the model of mutation at a given locus. For example, the distribution of allele frequencies expected for loci evolving under the stepwise mutation model (SMM; Ohta and Kimura 1973) may have a slightly lower proportion of alleles at low frequency than the distribution expected for the infinite allele model of mutation (IAM; Kimura and Crow 1964). However, alleles at low frequency (<0.1) are always expected to be more abundant than alleles at intermediate frequency, regardless of the mutation rate and model (Nei et al. 1976). Low frequency alleles are typically far more abundant than alleles at intermediate frequency in allozyme datasets from nonbottlenecked natural populations (Chakraborty et al. 1980).

The expected distribution of allele frequencies in a recently bottlenecked population has not been thoroughly studied, although numerous authors have reported that alleles at low frequency are expected to be lost rapidly during a bottleneck (Allendorf 1986; Denniston 1978; Nei et al. 1976; Maruyama and Fuerst 1985; Watterson 1984). Furthermore, no one has developed a method for identifying recently bottlenecked populations based on a distortion of allele frequency distributions (but see the quantitative method of Cornuet and Luikart, 1996). We present a qualitative graphical method for identifying bottlenecked populations from distributions of allele frequencies and evaluate the performance of the method using computer simulations and 90 datasets from natural populations.

Three questions we address here are as follows: (1) How are bottlenecks expected to distort the distribution of allele frequencies at neutral loci? (2) Is the expected distortion usually apparent in empirical datasets from bottlenecked natural populations? (3) How small a bottleneck is required to cause a distortion in allele frequencies that is likely to be detectable (power > 0.80) by the graphical method when using approximately 10 microsatellite loci? Questions 1 and 3 are addressed using Monte Carlo computer simulations.

If the graphical method is to be useful for detecting bottlenecks, both the simulations and empirical datasets from known bottlenecked natural populations should reveal a characteristic distortion in the distribution of allele frequencies. Furthermore, datasets from nonbottlenecked natural populations should not reveal this distortion, but rather should have a large proportion of alleles at low frequency, as expected in populations near mutation-drift equilibrium.

Methods

The Graphical Method

The graphical method consists of grouping alleles from a sample of many polymorphic loci (at least five loci) into each of 10 allele frequency classes and then plotting a frequency histogram. The 10 allele frequency classes are 0.001–0.100, 0.101–0.200, 0.201–0.300, etc. For the following discussions we define low- and high-frequency allele classes as 0–0.100 and 0.901–1.00, respectively. We define intermediate frequency classes as those eight classes between 0.101 and 0.900. This classification system is arbitrary but

useful for the qualitative graphical assessment of allele frequency distributions. We group alleles into only 10 allele frequency classes because if more than 10 classes are used, a meaningful assessment of the distribution of allele frequencies would often not be possible, because too few alleles exist in most empirical datasets, especially datasets from bottlenecked populations. The graphical method concludes that a population has been recently bottlenecked if fewer alleles are found in the low frequency class than in one or more intermediate frequency classes (see below).

Simulations

We conducted Monte Carlo computer simulations to determine the expected distribution of allele frequencies in a recently bottlenecked population. To determine the expected distribution, we calculated and graphed the mean number of alleles in each of 10 allele frequency classes after 500 bottleneck simulation replicates. In our simulation model, the genotypes of individuals in the initial generation of each bottleneck replicate are generated by randomly sampling from an allele frequency distribution of a nonbottlenecked population (e.g., the distribution in Figure 1a). Genotypes of each subsequent generation are generated by simulating Mendelian inheritance and random mating between males and females (sex ratio 1:1). Loci are assumed to be selectively neutral. The model does not incorporate new mutations; however, the number of new mutations over a small number of generations (i.e., 2 multiplied by N_e generations) will be negligible (Maruyama and Fuerst 1985).

Power Analysis

Computer simulations were also used to determine the bottleneck size required to cause a distortion in allele frequencies that is likely to be detectable (power $>$ 0.80) when sampling 8–10 polymorphic microsatellite loci and 30 individuals. These simulations were conducted in the same way as those described above with two exceptions meant to make the simulations more realistic. First, the initial prebottleneck allele frequencies were obtained from actual data from 8 and 10 microsatellite loci from nonbottlenecked populations of brown bears and wolves, respectively (Western Brooks Range brown bear population, *Ursus arctos,* Craighead 1994; and Hinton wolf population, *Canis lupus,* Forbes and Boyd 1996). These datasets were chosen because they are among the largest published (appendix 2), and their distribution of allele frequencies conform well to the distribution expected for loci at mutation-drift equilibrium evolving under the stepwise mutation model (Figure 2e,g; Luikart G and Cornuet J-M, unpublished data). Second, random samples of 30 individuals were generated from the last generation of each bottleneck replicate to simulate the process of estimating allele frequencies from a sample, as is done in empirical studies of natural populations.

Empirical Datasets

We analyzed 9 microsatellite datasets and 10 allozyme datasets from natural populations thought to have been recently bottlenecked and isolated based on evidence from demographic, biogeographic, and/or independent molecular data (appendix 1). Some datasets were selected as representatives of numerous published datasets from a given species (e.g., common myna birds; Baker and Moeed 1987; Fleisher et al. 1991).

We analyzed 25 microsatellite datasets and 46 allozyme datasets from natural populations that are not known to have been recently bottlenecked (appendixes 2 and 3). These datasets were chosen because they include many individuals (at least 20, except for the Brookfield wombat population), many polymorphic loci (at least 5), and populations from relatively undisturbed habitats, for example, Pacific salmon (*Oncorhynchus* sp.) from remote areas without hatchery influences, bears (*Ursus* sp.), mountain sheep (*Ovis canadensis*), and wolves (*C. lupus*) from Alaska and Canada. However, it is difficult to be certain that these populations, or any natural populations, have not experienced a recent bottleneck because information seldom exists on a population's historical size and N_e.

Results

Bottleneck-Induced Distortions

Our simulations showed that the defining characteristic of the distribution of allele frequencies expected in a recently bottlenecked population is a mode-shifted distribution, that is, a distribution with fewer alleles in the low frequency class ($<$0.1) than in one or more intermediate frequency classes (e.g., 0.1–0.2). We characterize this bottleneck-induced distortion of the distribution of allele frequencies as a mode shift, because bottlenecks shift the mode from low frequency to an intermediate frequency (compare Figure 1a,b).

Data From Natural Populations

Both the allozyme and microsatellite datasets from recently bottlenecked natural populations often revealed a characteristic mode-shift distortion in the distribution of allele frequencies. That is, these datasets often had fewer alleles at low frequency than in one or more intermediate allele frequency class. For example, the bottlenecked population of Epping wombats had only one allele at low frequency, but nine alleles between the frequencies 0.4 and 0.5 (Figure 2c). In total, 13 of 19 datasets from bottlenecked natural populations revealed a mode-shift distortion (appendix 1; Figure 2a–l, open bars).

Allozyme datasets from nonbottlenecked natural populations usually had a large proportion of alleles at low frequency, and thus had an allele frequency distribution with a mode in the low frequency class (Figure 2i–l, black bars; see also distributions from 138 populations in Chakraborty et al. 1980). Only one of the 46 allozyme datasets from nonbottlenecked populations revealed a mode-shift distortion in which alleles at intermediate frequencies were more abundant than alleles at low frequency (Sun River population of mountain sheep; appendix 3). Microsatellite datasets from nonbottlenecked populations also had a large proportion of rare alleles (Figure 2a–g, black bars; see also Allen et al. 1996; England et al., in press; and Roy et al. 1995). Only one (*M. domestics*-3) of the 25 microsatellite datasets from nonbottlenecked populations revealed a mode-shifted distribution of allele frequencies (appendix 2). Analyses of empirical datasets suggest that the graphical method is not likely to incorrectly identify a nonbottlenecked population as a recently bottlenecked population.

Simulations and Power Analysis

To further determine if the qualitative graphical method is likely to detect mode-shifted distributions in nonbottlenecked populations, we used computer simulations to generate samples of 20 to 30 individuals from allele frequency data published from a nonbottlenecked population of brown bears (Western Brooks Range; Craighead 1994). In "computer samples" of 20, 25, and 30 individuals from the bear dataset, the proportion of 500 samples revealing a mode-shifted distribution were 0.05, 0.010, and 0.006, respectively.

Our computer simulation power analysis suggested that bottlenecks of size

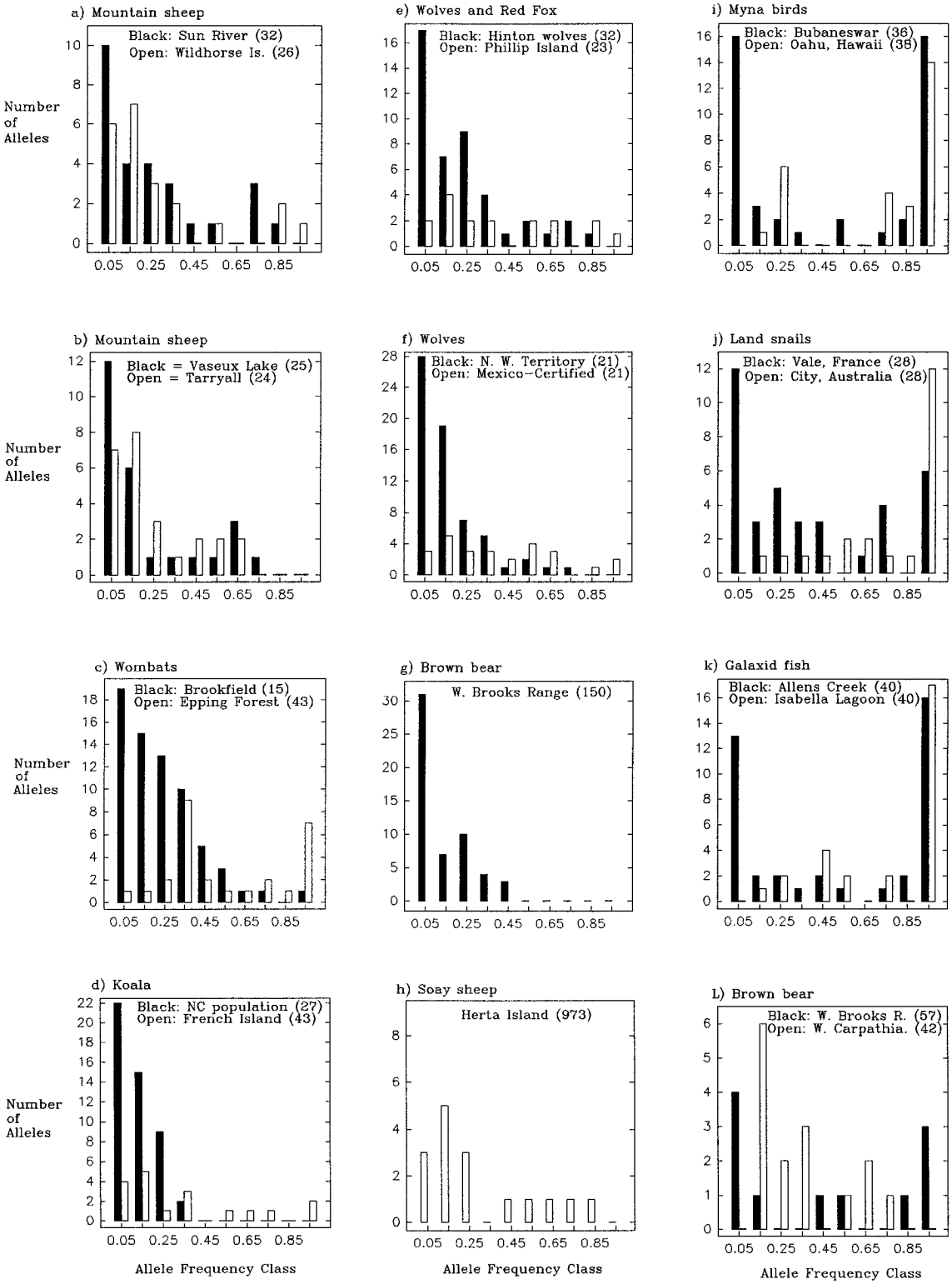

Figure 2. (a–l) Allele frequency distributions from nonbottlenecked (black bars) and bottlenecked natural populations (open bars). Figures h–l are from allozyme data; the others are from microsatellites. Each figure (except g and h) shows a distribution from both a nonbottlenecked and a bottlenecked population from the same species or two related species. The number in parentheses is the sample size of individuals.

ranging up to 20 individuals have a reasonably high probability of being detected (0.78) using the qualitative graphical test for mode shift, and samples of 8 microsatellite loci and 30 individuals (Figure 3). In these simulations, the prebottleneck allele frequencies for the eight loci were obtained from the Western Brooks Range brown bears (Figure 2g, black bars; Craighead 1994). Power estimates slightly higher than those from the bear data were achieved using data from 10 microsatellite loci from the Hinton population of wolves (Figure 2e, black bars; Forbes and Boyd 1996).

Discussion

Our simulations have shown that population bottlenecks are expected to cause a mode-shift distortion in the distribution of allele frequencies at neutral loci such that alleles in the low frequency class (<0.1) become less abundant than alleles in one or more intermediate frequency classes (e.g., 0.1–0.2). We have illustrated a qualitative graphical method of analyzing allele frequency data that can help detect mode-shift distortions and thereby help identify recently bottlenecked populations.

The main advantage of this method is that no reference population or data on historical levels of genetic variation is needed to determine if a population has been recently bottlenecked. For example, based only on contemporary allele frequency distributions and without comparative data on levels of genetic variation in nonbottlenecked reference populations, one can conclude that the Epping Forest wombats have recently suffered a genetic bottleneck, loss of genetic variation (i.e., at least rare alleles), and possibly the fixation of deleterious alleles (Figure 2c, open bars).

Even if reference populations are available, researchers testing for bottlenecks should analyze distributions of allele frequencies along with traditional indices of genetic variation such as heterozygosity (*H*), mean number of alleles per locus (*A*), and proportion of polymorphic loci (*P*). Traditional indices of genetic variation can remain high after a bottleneck and thus fail to detect a bottleneck. For example, in the bottlenecked population of brown bears from the western Carpathian Mountains, the mean number of alleles per locus is three (appendix 1, population 18; Hartle and Hell 1994). This *A* is high in comparison to allozyme data from other large mammals, including nonbottlenecked populations of brown bears from North America (appendix 3; Figure 2l, black bars). Although *A* is still high in the western Carpathian bears, the distribution of allele frequencies is mode-shift distorted as expected in this recently bottlenecked population (Figure 2l, open bars).

Another advantage of the graphical method is that it is most likely to detect the type of bottlenecks that are most likely to be harmful, that is, recent small bottlenecks. The smaller the bottleneck, the more likely it will increase the frequency of deleterious alleles and cause inbreeding depression and loss of genetic variation. Fortunately the smaller the bottleneck, the more likely it will be detectable by tests for distortions of allele frequency distributions (Figure 3, bottleneck size 4 versus 20). Recent bottlenecks are important to detect because recently bottlenecked populations are unlikely to have had time to adapt to the genetic and demographic problems often caused by bottlenecks.

We define "recent" as within the past several dozen generations. This definition is based on the time during which a distortion of allele frequency distributions is likely to be detectable. A bottleneck is

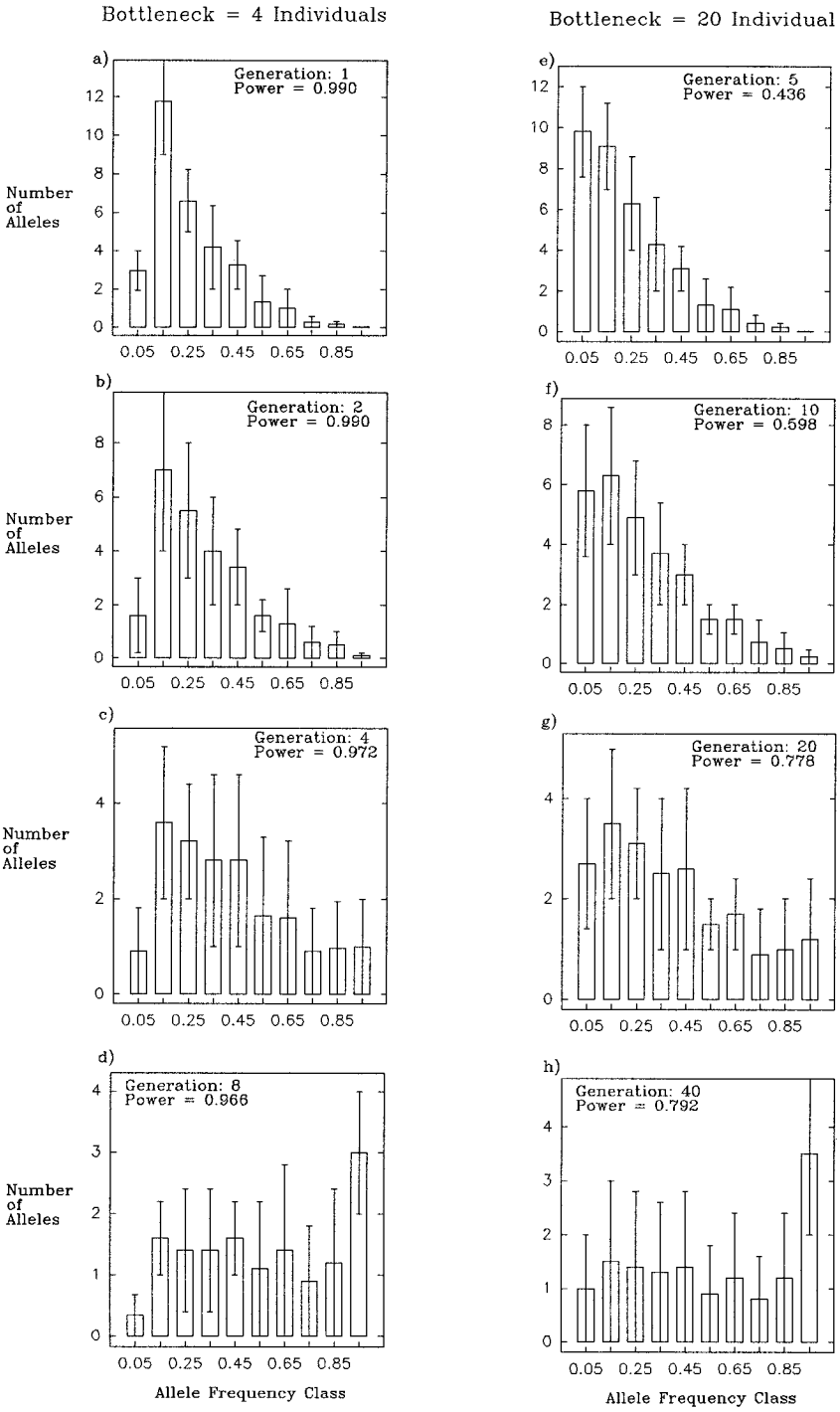

Figure 3. Distributions of allele frequencies in a sample of 30 individuals and 8 microsatellite loci from "computer populations" bottlenecked to 4 individuals and 20 individuals for each of four different generation times. Open bars represent the mean number of alleles in each allele frequency class, calculated from 500 bottleneck simulation replicates. Error bars show the approximate 20–80 percentile range of the number of alleles found in each frequency class over 500 simulations. Power is the proportion of 500 bottleneck replicates that revealed a mode-shift distribution of allele frequencies in the postbottleneck sample of 30 individuals.

likely to be detectable for only 40 to 80 generations, assuming that the maximum bottleneck size likely to be detectable is approximately $N_e = 20$ (Figure 3), and that bottlenecks are detectable for only approximately $2N_e$ to $4N_e$ generations until genetic drift and new mutations begin to reestablish mutation-drift equilibrium (Cornuet and Luikart, 1996; Maruyama and Fuerst 1985; Nei and Li 1976).

Houlden et al. (1996) and Huettle et al. (1980) have reported "mode shifts" (called "bimodal" distributions by these authors) in allele frequency distributions from bottlenecked natural populations of Mediterranean fruit flies (*Ceratitis capitata*) and Australian koalas (*Phascolarctos cinereus*), respectively. These authors hypothesized that a mode shift may typically result from a bottleneck and that the mode should shift to an intermediate allele frequency between 0.4 and 0.6 or 0.3 and 0.7. However, our simulations suggest that the mode at intermediate frequency is usually expected to occur between the frequencies 0.1 and 0.2 (Figure 1b and 3). Furthermore, the mode at intermediate frequency is only expected to occur between the frequencies 0.2 and 0.5 when the bottleneck is both smaller than approximately eight breeding individuals and persists for several generations (data not shown).

The degree of mode shift is expected to increase with bottleneck severity. Thus the degree of mode shift may be useful for inferring the approximate severity of a recent bottleneck. However, reliable inferences about the approximate severity of a bottleneck may require many polymorphic loci (>10) because there is substantial variability among the different distributions of allele frequencies that can actually result from a bottleneck (see error bars in Figure 3).

It is important to note that genetic bottlenecks of less than 20 individuals are not unrealistically small for many wild and captive populations because the genetically effective size (N_e) of a population is often only 10–20% of a population's census size, and occasionally a much smaller percentage (Briscoe et al. 1992; Frankham 1995b). Consequently even populations with a large census size in national parks, wildlife reserves, or fish hatcheries could experience a severe genetic bottleneck in the absence of a demographic bottleneck. For example, if only a few males mate with all the females in a large population, a genetic bottleneck can occur without a demographic bottleneck. Analyses of allele frequency distributions can help detect these "cryptic" genetic bottlenecks in populations thought to have a large N_e based on demographic data.

Assumptions

When analyzing allele frequency distributions to test for bottlenecks, it may be necessary to assume that the test population is random mating, has no substructure, has no recent immigration, loci are neutral, and that sampling is representative of the population. These assumptions and the consequences of violating the assumptions have been discussed by Cornuet and Luikart (1996). It is worth reiterating here that tests for Hardy–Weinberg proportions may detect violations of the above assumptions. Loci not in Hardy–Weinberg proportions should be excluded or used only with caution. We tested for bottlenecks with and without loci deviating from Hardy–Weinberg proportions in the datasets from which genotype frequency data was available, but it made no difference in the test results.

Violating the assumption that loci are selectively neutral could cause nonbottlenecked populations to appear to have been recently bottlenecked, if the type of selection is heterozygote advantage or balancing selection. Strong balancing selection could maintain alleles at intermediate frequencies and thereby reduce the proportion of alleles at low frequency and generate a mode-shift distortion. Researchers have reported evidence of balancing selection at allozyme loci in several of the nonbottlenecked populations analyzed in this study [American oysters (*Crassostrea virginica*), Karl and Avise 1992; and Atlantic cod (*Gadus morhua*), Pogson et al. 1995]. However, the graphical method detected no evidence of mode-shift distortion in any of the oyster or cod populations (appendix 3).

Performance of Method

It is difficult to evaluate the performance of methods for detecting bottlenecks using data from natural populations because few datasets with many polymorphic loci exist from bottlenecked populations that are isolated and have a well-documented history of bottleneck size and duration. Nevertheless, it is important to evaluate the behavior of genetic markers and the performance of bottleneck tests in the few appropriate datasets available from natural populations.

Bottlenecked Populations

Simulations and empirical datasets both show that the qualitative graphical method often identifies recently bottlenecked populations. Moreover, the datasets with the largest proportion of alleles at intermediate frequency are from populations in which bottlenecks have been the most severe, the most well-documented, and for which the most polymorphic loci were analyzed (see the Epping Forest population, Figure 2c, open bars). We note that an unpublished microsatellite dataset from the severely bottlenecked Bison Range population of mountain sheep also shows a strongly mode-shifted distribution of allele frequencies similar to that of the Epping wombats (appendix 1; Hogg J and Forbes S, personal communication).

The six bottlenecked populations that did not have a mode-shifted distribution (appendix 1) may not have a distorted distribution for the following reasons: (1) the bottleneck was not recent or small enough to be detectable, (2) not enough polymorphic loci and/or individuals were sampled to have sufficient power for detecting the bottleneck, (3) the individuals sampled were not representative of the bottlenecked population, (4) a demographic bottleneck occurred but not a genetic bottleneck (i.e., $N_e \gg N$-census), and (5) the bottlenecked population is not completely isolated and contains genes from immigrants that have obscured the genetic effects of the bottleneck.

The bottlenecked populations of Nepal rhinoceros and Sidney myna birds may not have a mode-shifted distribution because the bottlenecks were not small (i.e., 60–80 individuals and 100 individuals, respectively). The bottlenecked population of Illinois-WHIT tree sparrows may not have a mode-shifted distribution because the bottleneck occurred long ago (i.e., more than 100 generations ago; appendix 1).

If analyses of allele frequency distributions fail to detect a mode-shifted distribution of allele frequencies, one should not conclude that a population has not been bottlenecked; one can only conclude that a bottleneck is not *likely* to have occurred in the recent past. Although a mode-shifted distribution is likely to be detectable (power = 0.78) after a bottleneck of less than approximately 20 breeding individuals, it will not be detected approximately 22% of the time when 8 to 10 polymorphic microsatellite loci are screened. Furthermore, it may take 5 to 10 generations for bottlenecks of 20 breeders

to generate a mode-shifted distribution (Figure 3e,f). Consequently a mode-shifted distribution occasionally may not be detected even though a population has been recently bottlenecked.

Nonbottlenecked Populations

Both the simulations and empirical datasets suggested that the qualitative graphical method is not likely to incorrectly identify a nonbottlenecked population as a recently bottlenecked population. Only 2 of the 71 datasets from "nonbottlenecked" populations revealed a mode-shifted distribution of allele frequencies that is characteristic of bottlenecked populations. The simulations showed that sample sizes of 20 to 30 individuals are unlikely to suggest that a nonbottlenecked population has been recently bottlenecked. Nonetheless, small samples are likely to miss alleles at low frequency (Sjogren and Wyoni 1994) and thus cause allele frequency distributions to resemble those from a bottlenecked population. Consequently we recommend sampling more than 30 individuals to avoid mistakenly identifying a nonbottlenecked population as a recently bottlenecked population.

Conclusions

Empirical data and computer simulations show that population bottlenecks cause a characteristic mode-shift distortion in the distribution of allele frequencies at selectively neutral loci. This distortion is often detectable by the qualitative graphical method. Eight to ten microsatellite loci provide approximately an 80% probability (power) of detecting a recent historical bottleneck of fewer than 20 breeding individuals. The graphical method is unlikely to mistakenly identify a nonbottlenecked population as a bottlenecked population if at least 30 individuals are sampled.

The graphical method for detecting distortions in the distribution of allele frequencies can identify recently bottlenecked populations without use of reference populations or information about historical population size or levels of genetic variation. Even when reference populations are available for comparison, researchers testing for recent bottlenecks or loss of genetic variation should analyze the distribution of allele frequencies in addition to traditional indices of genetic variation. Analyses of allele frequency distributions may identify bottlenecked populations when traditional indices of genetic variation do not.

Analyses of allele frequency distributions can help detect "cryptic" genetic bottlenecks in which N_e has been severely reduced in the absence of a reduction of a population's census size. The graphical method provides a useful tool for identifying recently bottlenecked populations that may be of concern for conservation biology.

Appendix 1. Census sizes and sample sizes of loci and individuals in 19 datasets from bottlenecked populations

Species and populations	Number of polymorphic loci/alleles sampled	Mean number of individuals sampled per locus	Historical population census size/date	Source of genetic and census data
Mountain sheep (*Ovis canadensis*)				
1. Wildhorse Island, Montana	6/22	26	8 founders/1947 (transplanted from Sun River, Montana, although 2 of the 8 may be from a different source), 90/1954, 130/1964, 309/1971, 205/1972, 200/1994	Microsatellites Luikart G, u.d.; Matthews 1973
2. Tarryall, Colorado	6/25	24	900/pre-1952, 44/1953, 100/1970, 150/1981, 200/1988	Microsatellites Luikart G, u.d.; Buchner 1960; Bailey J, personal communication
3. Bison Range, Montana U.S.A.	7/20	23	12 founders/1921 (transplanted from Banff near Sheep River, Canada), 90/1929, 8/1939, 12/1950, 50/1984	Microsatellites Forbes S, Hogg J, u.d.; National Bison Range census records
Wombats (Epping = *Lasiorinyus krefftii*)				
4. Epping Forest, Queensland, Australia	9/31	43	20–30/1980, 70/1994	Microsatellites Taylor 1995
Red fox (*Vulpes vulpes*)				
5. San Remo, Victoria, Australia	7/28	22	5 founders/1870, however, other undocumented introductions of *V. vulpes* may have occurred. The population has grown and spread across much of Australia	Microsatellites Lade et al. 1996
6. Phillip Island, Victoria, Australia	7/17	23	Unknown number of founders from the Australian mainland population described in 5 above	See 5

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Appendix 1. Continued

Species and populations	Number of polymorphic loci/alleles sampled	Mean number of individuals sampled per locus	Historical population census size/date	Source of genetic and census data
Koalas (*Phascolarctos cinereus*)				
7. French Island, Australia	4/16	43	As few as 2–3/around 1900	Microsatellites Houlden et al. 1996
Wolves (*Canis lupus*)				
8. Mexican-certified Mexico	10/26	21	4 founders/1984 (from a remnant population of the endangered Mexican wolf)	Microsatellites Garcia-Moreno et al. 1996
Soay sheep (*Ovis aries*)				
9. Herta Island, Scotland	6/23	662	107 founders/1932 (introduced from Soay Island), size fluctuates between 600–1,500 every 3–5 years	Microsatellites Bancroft et al. 1995
10. Herta Island, Scotland	5/16	973	Same as in 9	Allozymes Bancroft et al. 1995
Common myna bird (*Acridotheres tristis*)				
11. Oahu, Hawaii	7/28	38	About 100 founders/1982 (introduced from India), now abundant on all Hawaiian islands	Allozymes Fleischer et al. 1991
12. Sidney, New South Wales, Australia	9/32	42	About 100 founders/1862 (introduced from India), now widely abundant in Sydney and surrounding cities	See 11
Land snails (*Thebia pisana*)				
13. City, Australia	5/23	28[a]	Unknown number introduced in the 1890s	Allozymes Johnson 1988
14. Cott, Australia	6/25	28[a]	Same as in 13	See 13
Galaxid fish (*Galaxias truttaceus*)				
15. Isabella Lagoon, Tasmania	5/28	40	Bottleneck inferred from mitochondrial DNA data, population became landlocked 3000–7000 years ago	Allozymes Ovenden and White 1990
Eurasian tree sparrow (*Passer montanus*)				
16. Illinois-WOOD	8/23	24	20/1870 (introduced from Europe)	Allozymes St. Louis and Barlow 1998
17. Illinois-WHIT	9/25	52	Same individuals as in 16	See 16
Brown bear (*Ursus arctos*)				
18. Western Carpathians, Romania	5/15	57	40/1932, 700/1995 Isolated from other populations since the late 1800s	Allozymes Hartle and Hell 1994
Rhinoceros (*Rhinoceros unicornus*)				
19. Chitwan Valley, Nepal	10/20	22	>1,000/1950, 60–80/1962, >251/1988	Allozymes Dinerstein and McCracken 1990

All data sets had a mode-shifted distribution except 5, 9, 12, 14, 17, and 19. u.d. = unpublished data; n.r. = not reported.
[a] Approximately 28, exact number not reported.

Appendix 2. Twenty-five microsatellite datasets from populations not known to have been recently bottlenecked

Species and population	Number of polymorphic loci analyzed	Average number of individuals sampled per locus	Reference
Mountain sheep			
Sun River	6	32	a
Vaseux Lake	6	25	a
Sheep River	8	50	b
Wolves			
Hinton	10	32	c
N.W. Territory	10	21	d
Coyotes (*Canis latrans*)			
California	10	22	d
Brown bears			
W. Brooks Range	8	152	e
Polar bears (*Ursus maritimus*)			
W. Hudson Bay	8	30	f
Davis Strait	8	26	f
N. Beaufort	8	30	f
S. Beaufort	8	22	f
Wombats (*Lasiorhinus krefftii*)			
Brookfield	14	16	g
Koalas			
Gold Coast	6	27	h
Field mice (*Mus musculus* and *M. domesticus*)			
M. domesticus-3	6	24	k
M. musculus-7.92	5	24	k
Chimpanzees (*Pan troglodytes*)			
Gombe Kasakela	8	36	q
Humans (*Homo sapiens*)			
Sardinia	10	46	j
Egypt	10	46	j
Kachari	6	40	l
New Guinea	6	39	l
Gray seals (*Halichoerus grypus*)			
Isle of May (adults)	8	35	m
North Rona (adults)	8	176	m
Fruit flies (*Drosophila melanogaster*)			
Tyrell	8	68	h
Bumble bees (*Bombus terrestris*)			
Corsica	7	21	i
Sardinia	7	22	i

Only one dataset revealed a mode-shifted distribution (field mice, *M. domesticus*-3 population). a = Luikart G, unpublished data, available upon request; b = Hogg J and Forbes S, personal communication; c = Forbes and Boyd 1996; d = Roy et al. 1994; e = Craighead 1994; f = Paetkau et al. 1995; g = Taylor et al. 1994; h = England et al., in press; i = Estoup et al. 1996; j = Di Rienzo et al. 1994; k = Dallas et al. 1995; l = Deka et al. 1991; m = Allen et al. 1996; n = Waits et al. 1996; q = Morin et al. 1994.

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Appendix 3. Forty-six allozyme datasets from populations thought *not* to have been recently bottlenecked

Species and population	Number of polymorphic loci analyzed	Mean number of individuals analyzed per locus	Reference
Mountain sheep			
Sun River	5	29	aa
Wolves			
Tuktoyaktuk	5	93	bb
Brown bear			
Western Brooks Range	5	42	cc
Common myna birds			
Bophal	15	40	dd
Loknow	16	40	dd
Bhubaneswar	15	36	dd
Eurasian tree sparrows			
Germany	12	30	ee
Sweden	25		ee
Minke whales (*Balenoptera acutorostrata*)			
MKC	9	45	ff
MBC	12	190	ff
Bryd whales (*Balenoptera edeni*)			
BMA	6	100	ff
BJA	6	118	ff
Galaxid fish			
Allens Creek	12	40	gg
Fortesue Lagoon	10	42	gg
Pink salmon (*Oncorhynchus gorbuscha*)			
Ivashka	21	75	hh
Kik-chik	16	78	hh
Pymta	23	79	hh
Arman	21	79	hh
Chum salmon (*O. keta*)			
Anadyr	26	100	ii
Ola	29	80	ii
Kamchatka-b	21	39	ii
Sockeye salmon (*O. nerka*)			
Skilak	13	50	jj
Yenta	7	50	jj
Dalnee 89-90	7	250	kk
Atlantic cod (*Gadus morhua*)			
Cod-E	8	95	ll
Cod-F	7	96	ll
Cod-I	5	98	ll
Cod-G	7	96	ll
Crabs (*Halice tridans* and *Chiromantes dehaani*)			
H. tridans-2	6	39	mm
H. tridans-3	7	40	mm
C. dehaani-2	8	39	mm
C. dehaani-1	5	23	mm
Land snails			
Vale, France	11	28	nn
CNRS, France	11	28	nn
American oysters (*Crassostrea virginica*)			
Cape Cod, Massachusetts	5	90	oo
Charleston, South Carolina	5	100	oo
Bay Grabe, Louisiana	5	88	oo
Brownsville, Texas	5	97	oo
Milk fish (*Chanos chanos*)			
Oahu	7	60	pp
Tarawa	10	38	pp
Christmas Island	6	47	pp
New Zealand conifers (*Halocarpus bidwillii*)			
Pop-14	7	38	qq
Pop-16	5	76	qq
Pop-2	5	40	qq
Pop-6	5	40	qq
Scots pine (*Pinus sylvestris*)			
Yllastunturi	12	44	rr

Only one dataset revealed a mode-shifted distribution (Mountain sheep, Sun River population). aa = Knudsen K and Allendorf FW, personal communication; bb = Kennedy et al. 1991; cc = Knudsen K and Allendorf FW, personal communication; dd = Baker and Moeed 1987; ee = St. Louis and Barlow 1988; ff = Wada and Numachi 1991; gg = Ovenden and White 1989; hh = Shaklee and Varnavaskya 1994; ii = Winans et al. 1994; jj = Allendorf FW and Knudsen K, personal communication; kk = Varnavaskya et al. 1994; ll = Mork et al. 1985 (these loci were suspected to be under balancing selection when they were compared to nuclear DNA RFLP loci, Pogson et al. 1995); mm = Irawan et al. 1993; nn = Johnson 1988; oo = Buroker 1983—we used only the five loci used by Karl and Avise (1992) and suspected by them to be under balancing selection; pp = Winans 1980; qq = Billington 1991; rr = Savolainen and Hedrick 1995.

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