Empirical Evaluation of a Test for Identifying Recently Bottlenecked Populations from Allele Frequency Data

GORDON LUIKART*‡ AND JEAN-MARIE CORNUET†

*Division of Biological Sciences, University of Montana, Missoula, MT 59812, U.S.A.
†Laboratoire de Modelisation et de Biologie Evolutive, I.N.R.A./U.R.L.B., 488 rue Croix de Lavit 34090 Montpellier Cedex, France

Introduction

Identifying recently bottlenecked populations (populations severely reduced in size) is important because bottlenecks can increase demographic stochasticity, rate of inbreeding, loss of genetic variation, and fixation of deleterious alleles and, thereby, reduce adaptive potential and increase the probability of population extinction (Frankel & Soulé 1981; Lande 1988, 1994; Leberg 1990; Hedrick & Miller 1992; Mills & Smouse 1994; Frankham 1995*a*, 1995*b*; but see Bryant et al. 1986; Goodnight 1987). Unfortunately, it is usually difficult to determine if a population has recently experienced a bottleneck because historical population sizes and levels of genetic variation are seldom known.

We developed a statistical test (a sign test for heterozygosity excess) for detecting recent historical bottlenecks using allele frequency data (Cornuet & Luikart 1996). The test requires no data on historical population sizes or levels of genetic variation; it requires only measurements of allele frequencies from 5 to 20 polymorphic loci in a sample of approximately 20-30 individuals. The test has reasonable statistical power when applied to allele frequency data sets generated by computer simulations (Cornuet & Luikart 1996). The performance of the test, however, must be evaluated by means of empirical data from natural populations before it can be used with confidence.

Our objectives were to (1) explain to conservation biologists the principle of the sign test for detecting heterozygosity excess and (2) evaluate the reliability of the test by analyzing 56 allozyme and 37 microsatellite data sets from bottlenecked and nonbottlenecked natural populations.

Principle of the Test

In natural populations allele number and heterozygosity at selectively neutral loci result from an equilibrium between mutation and genetic drift. The heterozygosity expected at a locus in an equilibrium population (H_{eq}) can be calculated from the number of alleles observed and the sample size of individuals, assuming neutrality and mutation-drift equilibrium. In nonbottlenecked populations that are near mutation-drift equilibrium, the expected heterozygosity (H_{eq}) will equal the measured Hardy-Weinberg equilibrium heterozygosity (H_e). But if a population has suffered a recent bottleneck, the mutation-drift equilibrium is transiently disrupted and the heterozygosity measured at a locus (H_e) will exceed the heterozygosity (H_{eq}) computed from the number of alleles sampled (Watterson 1984; Maruyama & Fuerst 1985; Cornuet & Luikart 1996).

Bottlenecks generate a "heterozygosity excess" because alleles are generally lost faster than heterozygosity during a bottleneck (Fig. 1). Alleles are lost faster than heterozygosity because rare alleles are lost rapidly during a bottleneck and because they have little effect on heterozygosity (Hedrick et al. 1986). Thus, many alleles can be lost without much reduction in heterozygosity. The proportion of allelic diversity retained was calculated as $[(n' - 1) / (n - 1)]$ (Fig. 1), where n' is the total number of alleles remaining and n is the original number of alleles in the prebottleneck population (Allendorf 1986). The curves for mean H_e and allelic diversity were generated from 500 Monte Carlo simulations in which the first bottleneck generation was generated by randomly sampling from microsatellite

‡*Address correspondence to G. Luikart, Laboriatoire de Biologie des Populations d'Altitude, CNRS UMR 5553, Université Joseph Fourier, BP 53 F-38041, Grenoble Cedex 9, France*
Paper submitted October 28, 1996; revised manuscript accepted April 3, 1997.

Conservation Biology, Pages 228–237
Volume 12, No. 1, February 1998

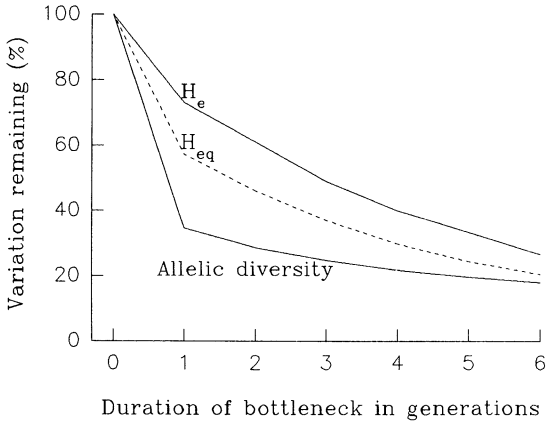

Figure 1. Loss of mean heterozygosity (H_e) and alleles (allelic diversity) at eight microsatellite loci during a bottleneck of two individuals. Allelic diversity is lost faster than heterozygosity, causing a heterozygosity excess ($H_e > H_{eq}$). The H_{eq} is the heterozygosity expected in a population at mutation drift equilibrium, given the number of alleles observed in the bottlenecked population. The H_{eq} was computed assuming that loci evolve under the stepwise model of mutation. The distance between the curves for H_e and H_{eq} represents the expected magnitude of the heterozygosity excess.

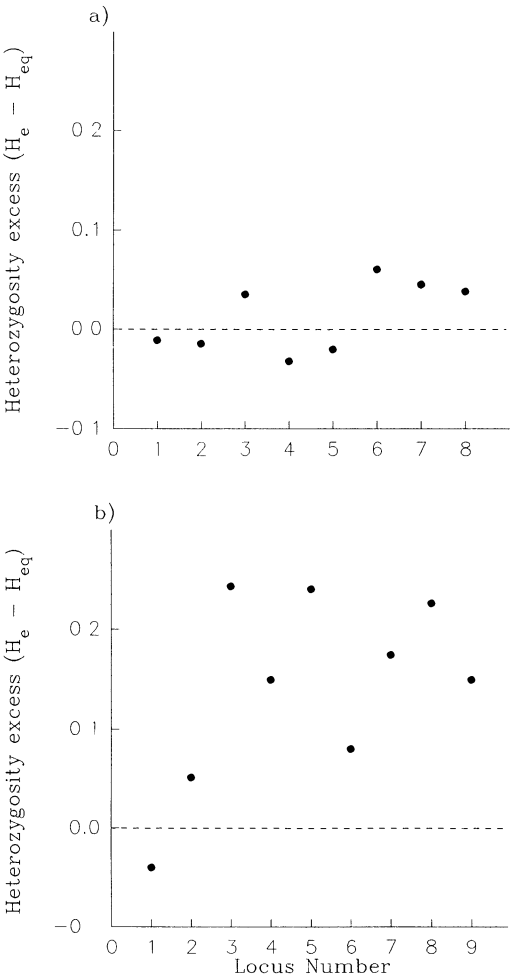

Figure 2. Magnitude of heterozygosity excess observed at each of eight polymorphic microsatellite loci in the nonbottlenecked population of brown bears (Western Brooks Range; Craighead 1994) (a) and nine polymorphic microsatellite loci from the bottlenecked population of wombats (Epping Forest population; Taylor et al. 1994) (b). The horizontal dashed line represents the heterozygosity excess expected in an equilibrium population with loci evolving under the stepwise model of mutation. Points above the dashed line represent loci with a heterozygosity excess; points below are loci with a heterozygosity deficiency.

allele frequencies from the large nonbottlenecked population of Western Brooks Range brown bears (*Ursus arctos*; Craighead 1994).

The bottleneck-induced heterozygosity excess is transient and is likely to be detectable only for a short time, approximately 0.2–4.0 N_e generations, until a new equilibrium between mutation and drift is reached at the new N_e (N_e is the bottleneck effective size; Cornuet & Luikart 1996). Thus, only bottlenecks that have occurred in the recent past (less than 4 N_e generations ago) are likely to be detectable by the sign test for heterozygosity excess. This window of time is approximate and depends not only on N_e but also on factors such as the mutation rate and mutation model of the loci sampled (Cornuet & Luikart 1996). It also assumes an immediate and permanent bottleneck in population size.

In a nonbottlenecked, equilibrium population, approximately 50% of the loci sampled are expected to have a slight excess of heterozygosity ($H_e > H_{eq}$), and 50% will have a slight deficiency of heterozygosity ($H_e < H_{eq}$), resulting from genetic drift and sampling error (Fig. 2a). Recently bottlenecked populations are expected to have a majority of loci with a substantial excess of heterozygosity (Fig. 2b). The sign test (Cornuet & Luikart 1996) determines if a significant majority of loci in a population have a heterozygosity excess, and thus if a population appears to have been recently bottlenecked.

Tests for heterozygosity excess should not be confused with tests for Hardy-Weinberg proportions. Tests for Hardy-Weinberg proportions compare the observed proportion of heterozygotes (H_o) to the heterozygosity expected (H_e) when a population is in Hardy-Weinberg proportions. The test for heterozygosity excess compares H_e to the heterozygosity (H_{eq}) expected at mutation-drift equilibrium in a sample that has the same size and the same number of alleles as the sample used to measure H_e.

It is important to note that the calculation of H_{eq} depends on the model of mutation used to analyze the loci being studied (Cornuet & Luikart 1996). We used two

models of mutation to calculate H_{eq}: the strict one-step stepwise mutation model (SMM, Ohta & Kimura 1973), and the infinite allele model (IAM, Kimura & Crow, 1964). The SMM and IAM represent two extreme models of mutation (Chakraborty & Jin 1992). Under the strict SMM, mutations change the state of an allele by one step forward or backward with equal probability. Thus, the SMM allows mutation to existing states, whereas under the IAM mutations always result in new, nonexisting states. Most loci probably evolve according to a model intermediate between the IAM and SMM (Di Rienzo et al. 1994). Consequently, the actual expected equilibrium heterozygosity (H_{eq}) for a given locus probably lies between the H_{eq} values calculated by these two models. A copy of the computer program "Bottleneck," which conducts the sign test using both the IAM and SMM, is available from the authors.

Table 1. Number of data sets with a significant heterozygosity excess, with non-significant deviation from mutation-drift equilibrium expectations and with a significant heterozygosity deficiency (excess/equilibrium/deficiency) for recently bottlenecked and nonbottlenecked populations, under two models of mutation (SMM and IAM).

Bottleneck history and type of genetic markers analyzed	*Heterozygosity excess/ equilibrium/deficiency*	
	SMM[a]	*IAM*[b]
Bottlenecked		
Microsatellites	5/6/0	10/1/0
Allozymes	5/5/0	6/4/0
Nonbottlenecked		
Microsatellites	1/22/3	7/17/2
Allozymes	0/28/18	0/41/5

[a] *Stepwise mutaiton model.*
[b] *Infinite allele model (see text).*

Results

We conducted the sign test on 21 data sets (11 microsatellite and 10 allozyme data sets) from bottlenecked natural populations and on 72 data sets (26 microsatellite and 46 allozyme data sets) from nonbottlenecked natural populations (Appendices 1–3). The data set from the bottlenecked Epping Forest wombat population had eight loci with a heterozygosity excess and one with a heterozygosity deficiency, when either the IAM or the SMM was assumed (Fig. 2b, Appendix 1). This ratio (8:1) is significantly different from the expected ratio (1:1) for a nonbottlenecked, equilibrium population. In total, 5 of 11 microsatellite data sets and 5 of 10 allozyme data sets from bottlenecked populations revealed a significant heterozygosity excess under the SMM (Table 1). Under the IAM, 10 of 11 microsatellite data sets and 6 of 10 allozyme data sets revealed a significant heterozygosity excess. The data sets from bottlenecked populations that did not have a significant heterozygosity excess generally deviated toward an excess of heterozygosity ($H_e > H_{eq}$), as expected for bottlenecked populations (Appendix 1).

Only one of the 26 microsatellite data sets from nonbottlenecked populations revealed a significant heterozygosity excess ($p < 0.05$) under the SMM, suggesting that this population has been recently bottlenecked (Table 1). When the IAM was assumed, 7 of the 26 microsatellite data sets had a significant heterozygosity excess.

Interestingly, three of the microsatellite data sets from nonbottlenecked populations showed a significant *deficiency* of heterozygosity under the SMM, and two populations showed a significant heterozygosity deficiency under the IAM (Table 1). This suggests that these populations are not at mutation-drift equilibrium but instead have experienced a recent expansion in population size or perhaps a recent influx of rare alleles from genetically distinct immigrants.

None of the 46 allozyme data sets from nonbottlenecked populations had a significant heterozygosity excess (Table 1). But 18 populations under the SMM and 5 under the IAM revealed a significant heterozygosity *deficiency.* Finding a substantial number of allozyme data sets with a heterozygosity deficiency (an excess of alleles) agrees with the findings of Chakraborty et al. (1980).

Discussion

The assumptions of the sign test, and the consequences of violating the assumptions, have been discussed by Cornuet and Luikart (1996). It is worth reiterating here that, in tests for bottlenecks, loci that are not in Hardy-Weinberg proportions should be used only with caution because they could bias the test results. For example, a locus could deviate from Hardy-Weinberg proportions by having an excess of heterozygotes due to strong overdominance selection. Such a locus might also have a selection-induced heterozygosity excess and, therefore, should be used with caution in the sign test for heterozygosity excess. For the data sets studied here, excluding loci that were not in Hardy-Weinberg, proportions did not change the results of the sign test. Several data sets could not be tested for Hardy-Weinberg proportions, however, because only allele frequencies—not genotype frequencies—were published.

The sign test identified only approximately 50–75% of the recently bottlenecked natural populations. But several of the undetected bottlenecks were either not very severe (e.g., Yellowstone brown bears, Nepal rhinoceros, and perhaps Australian land snails) or not very recent (e.g., Common Mynas, European Tree Sparrows, and perhaps Kodiak brown bears; Appendix 1). The populations with a significant heterozygosity excess were generally those in which bottlenecks have been

the most severe and the best documented and for which the most polymorphic loci were analyzed (see the Bison Range and Epping Forest populations; Appendix 1). Consequently, we conclude that the sign test can help detect a recent bottleneck in natural populations, especially if the bottleneck was severe.

One reason some bottlenecked populations were not identified by the sign test may be that it often requires 10–20 polymorphic loci to have a reasonably high probability (power > 0.80) of detecting a recent bottleneck. For example, when loci evolve under the IAM, 30 individuals and at least 10 polymorphic loci are required to achieve power > 0.80 for detecting a 100-fold reduction in N_e (Cornuet & Luikart 1996). Of the data sets tested here, the one with the most polymorphic loci was from the Epping Forest wombats; it had only 9 polymorphic loci. Also, some bottlenecks were perhaps not detectable for the following reasons: not enough individuals were sampled to have sufficient statistical power for detecting the bottleneck, the individuals sampled were not representative of the bottlenecked population, or the bottlenecked population was not completely isolated and contained genes from immigrants (e.g., rare alleles) that have obscured the genetic effects of the bottleneck.

We conducted the sign test on data sets from nonbottlenecked populations because natural populations could develop an excess of heterozygosity even though a recent bottleneck has not occurred. Gene loci in natural populations may seldom be at mutation-drift equilibrium because of occasional fluctuations in population size and/or natural selection. Thus, it is important to determine if the sign test often detects a significant heterozygosity excess in empirical data sets from wild, nonbottlenecked populations.

Few data sets from "nonbottlenecked" populations had a significant heterozygosity excess (except for the microsatellite data sets tested under the IAM; Table 1). Some of the "non-bottlenecked" populations that did have a heterozygosity excess may actually have been recently bottlenecked. It is difficult to know a population's history because reliable information seldom exists on historical population size or N_e. Furthermore, because we conducted multiple tests, we expect some significant test results by chance alone. We tested 46 allozyme data sets and 26 microsatellite data sets and expected significant test results from 2.3 and 1.3 of the tests, respectively, assuming a Type I error rate of 0.05. Thus, we conclude that the sign test is not likely to suggest that nonbottlenecked populations have been recently bottlenecked.

Nonetheless, the sign test under the IAM may be prone to (wrongly) detecting heterozygosity excess in nonbottlenecked populations when microsatellite data are used, because microsatellites may tend to evolve under a model more similar to the SMM than the IAM (Table 1, nonbottlenecked populations; Shriver et al. 1993; Valdes et al. 1993). For any given data set, the IAM predicts a lower equilibrium heterozygosity (H_{eq}) than the SMM. Thus, the IAM is more likely to indicate that a significant heterozygosity excess exists ($H_e >> H_{eq}$). Consequently, to be statistically conservative one should use only the SMM when analyzing microsatellite data to test for recent bottlenecks.

But because the true model of mutation for most loci is probably intermediate between the IAM and SMM (Di Rienzo et al. 1994), we recommend using both models of mutation. For example, if the sign test under the IAM detects a significant heterozygosity excess, but the test under the SMM is only very close to being significant (as in the koalas from Kangaroo Island, $P_{SMM} = 0.065$, and the foxes from Phillip Island, $P_{SMM} = 0.079$; Appendix 1), then it seems reasonable and conservative from a conservation biology perspective to conclude that the population may have been recently bottlenecked.

Both models of mutation should also be used when the sign test is conducted on allozyme data. But allozyme data tend to fit the IAM better (Table 1, nonbottlenecked populations; Chakraborty et al., 1980). Consequently, the SMM may be unreasonably conservative from a conservation biology perspective in that the SMM may be unlikely to detect a bottleneck when a bottleneck has actually occurred.

In conclusion, the sign test for heterozygosity excess appears to reliably detect small bottlenecks in natural populations when at least 5 polymorphic loci and 20–30 individuals are analyzed. At least 10 polymorphic loci should be used to achieve high statistical power.

Acknowledgments

We thank P. England, S. Forbes, and F. W. Allendorf for reading earlier versions of this manuscript. Funding for analyses of mountain sheep samples was provided by the Boone and Crockett Club, the National Rifle Association, and the Division of Biological Sciences at the University of Montana. Mountain sheep samples were kindly provided by J. McCarthy, J. Cross, and J. Firebaugh (Montana Department of Fish Wildlife and Parks); O. Dyre, H. Langin, D. Low, D. Ward, P. Dialman, A. Fontana, A. Peat, A. Fontana, R. Demarchi, and B. Lincoln (British Columbia Ministry of Environment, Wildlife Branch); J. Jorgenson (Alberta Fish and Wildlife Service); J. Hogg (Craighead Wildlife/Wildlands Institute); M. Miller and M. Dunbar (Colorado Department of Fish and Wildlife); T. Spraker; and M. Festa-Bianchet.

Literature Cited

Allen, P. J., W. Amos, P. P. Popery, and S. D. Twice. 1996. Microsatellite variation in grey seals (*Halichoerus grypus*) shows evidence of genetic differentiation between two British breeding colonies. Molecular Ecology **4**:653–662.

Allendorf, F. W. 1986. Genetic drift and the loss of alleles versus heterozygosity. Zoo Biology **5:**181–190.

Baker, A. J., and A. Moeed. 1987. Rapid genetic differentiation and founder effect in colonizing populations of common Mynas (*Acridotheres tristis*). Evolution **41:**525–538.

Bancroft, D. R., J. M. Pemberton, S. A. Albon, A. Robertson, A. D. C. Maccoll, J. A. Smith, I. R. Stevenson, and T. H. Clutton-Brock. 1995. Molecular genetic variation and individual survival during populations crashes of an unmanaged ungulate population. Philosophical Transactions of the Royal Society of London Biological Sciences **347:**235–358.

Billington, H. L. 1991. Effect of population size on genetic variation in a dioecious conifer. Conservation Biology **5:**115–119.

Bryant, E. H., S. A. McCommas, and L. M. Combs. 1986. The effect of an experimental bottleneck upon quantitative genetic variation in the housefly. Genetics **114:**1191–211.

Buroker, N. E. 1983. Population genetics of the American oyster *Crassostrea virginica* along the Atlantic coast and the Gulf of Mexico. Marine Biology **75:**99–112.

Chakraborty, R., and L. Jin. 1992. Heterozygote deficiency, population substructure and their implications in DNA fingerprinting. Human Genetics **88:**267–272.

Chakraborty, R., P. A. Fuerst, and M. Nei. 1980. Statistical studies on protein polymorphism in natural populations. III. Distribution of allele frequencies and the number of alleles per locus. Genetics **94:**1039–1063.

Cornuet, J. M., and G. Luikart. 1996. Description and evaluation of two tests for detecting recent bottlenecks. Genetics **144:**2001–2014.

Craighead, L. F. 1994. Conservation genetics of grizzly bears. Ph.D. dissertation. Montana State University, Bozeman.

Dallas, J. F., B. Dod, P. Boursot, E. M. Prager, and F. Bonhomme. 1995. Population subdivision and gene flow in Danish house mice. Molecular Ecology **4:**311–320.

Deka, R., R. Chakraborty, and R. E. Ferrell. 1991. A population genetic study of six VNTR loci in three ethnically defined populations. Genomics **11:**83–92.

Dinerstein, E., and G. F. McCracken. 1990. Endangered one-horned Rhinoceros carry high levels of genetic variation. Conservation Biology **4:**417–422.

Di Rienzo, A., A. C. Peterson, J. C. Garza, A. M. Valdes, M. Slatkin, and N. B. Freimer. 1994. Mutational processes of simple sequence repeat loci in human populations. Proceedings of the National Academy of Sciences, USA **91:**3166–3170.

England, P. R., D. A. Briscoe, and R. Frankham. 1996. Microsatellite polymorphisms in a wild population of *Drosophila melanogaster*. Genetical Research Cambridge **67:**285–290.

Estoup, A., M. Solignac, J. M. Cornuet, and A. Scholl. 1996. Genetic differentiation of continental and island populations of *Bombus terrestris* (Hymenoptera, Apidae) in Europe. Molecular Ecology **5:**19–31.

Forbes, S. H., and D. K. Boyd. 1996. Genetic variation of naturally colonizing wolves in the central Rocky Mountains. Conservation Biology **10:**1082–1090.

Frankel, O. H., and M. E. Soulé. 1981. Conservation and evolution. Cambridge University Press, Cambridge, United Kingdom.

Frankham, R. 1995*a*. Inbreeding depression: a threshold effect. Conservation Biology **9:**792–799.

Frankham, R. 1995*b*. Conservation genetics. Annual Review of Genetics **29:**305–327.

Garcia-Moreno, J., M. C. Matocq, M. S. Roy, E. Geffen, and R. K. Wayne. 1996. Relationships and genetic purity of the endangered Mexican wolf based on analysis of microsatellite loci. Conservation Biology **10:**376–389.

Goodnight, C. J. 1987. On the effect of founder events on epistatic genetic variance. Evolution **41:**80–91.

Hedrick, P. W. 1995. Gene flow and genetic restoration: the Florida panther as a case study. Conservation Biology **9:**996–1007.

Hedrick, P. W., and P. S. Miller. 1992. Conservation genetics: techniques and fundamentals. Ecological Applications **2:**30–46.

Hedrick, P. W., P. F. Brussard, F. W. Allendorf, J. A. Beardmore, and S. Orzack. 1986. Protein variation, fitness, and captive propagation. Zoo Biology **5:**91–99.

Houlden, B. A., P. R. England, A. Taylor, W. D. Greville, and W. B. Sherwin. 1996. Low genetic variability of the koala (*Phascolarctos cinereus*) in south eastern Australia following a severe population bottleneck. Molecular Ecology **5:**269–282.

Huettle M. D., P. A. Fuerst, T. Maruyama, and R. Chakraborty. 1980. Genetic effects of multiple populations bottlenecks in the Mediterranean fruit fly. Genetics **94:**s47–s48.

Irawan, B., A. Kijima, and Y. Fujio. 1993. Genetic divergence among the three species of estuarine crab, *Helice tridens, H. japonica* and *Chiromantes dehaani*. Tohoku Journal of Agricultural Research **43:**101–109.

Johnson, M. S. 1988. Founder effects and geographic variation in the land snail *Thebia pisana*. Heredity **61:**133–142.

Karl, S. A., and J. C. Avise. 1992. Balancing selection at allozyme loci in oysters: implications from nuclear RFLPs. Science **256:**100–102.

Kennedy, P. K., M. L. Kennedy, P. L. Clarkson, and I. S. Liepins. 1991. Genetic variability in natural populations of the grey wolf. Canadian Journal of Zoology **69:**1183–1188.

Kimura, M., and J. F. Crow. 1964. The number of alleles that can be maintained in a finite population. Genetics **49:**725–738.

Lade, J. A., N. D. Murray, C. A. Marks, and N. A. Robinson. 1996. Microsatellite differentiation between Phillip Island and mainland Australian populations of the red fox *Vulpes vulpes*. Molecular Ecology **5:**81–87.

Lande, R. 1988. Genetics and demography in biological conservation. Science **241:**1455–1459.

Lande, R. 1994. Risk of population extinction from fixation of new deleterious mutations. Evolution **48:**1460–1469.

Leberg, P. L. 1990. Influence of genetic variability on population growth: implications for conservation. Journal of Fish Biology **37**(Supplement A)**:**193–195.

Luikart, G. 1997. Usefulness of molecular markers for detecting population bottlenecks and monitoring genetic change. Ph.D. dissertation. University of Montana, Missoula.

Maruyama, T., and P. A. Fuerst. 1985. Population bottlenecks and nonequilibrium models in population genetics. II. Number of alleles in a small population that was formed by a recent bottleneck. Genetics **111:**675–689.

Menotti-Raymond, M. A., and S. J. O'Brien. 1995. Evolutionary conservation of ten microsatellite loci in four species of Felidae. Journal of Heredity **86:**319–321.

Mills, S. L., and P. E. Smouse. 1994. Demographic consequences of inbreeding in remnant populations. American Naturalist **144:**412–431.

Mork, J., N. Ryman, G. Stahl, F. Utter, and G. Sundnes. 1985. Genetic variation in Atlantic cod throughout its range. Canadian Journal of Fisheries and Aquatic Science **42:**1580–1587.

Ohta, T., and M. Kimura. 1973. A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. Genetical Research Cambridge **22:**201–204.

Ovenden, J. R., and R. W. G. White. 1990. Mitochondrial and allozyme genetics of incipient speciation in a landlocked population of *galaxias truttaceus* (Pisces: Galaxiidae). Genetics **124:**701–716.

Paetkau, D., W. Calvert, I. Stirling, and C. Strobeck. 1995. Microsatellite analysis of population structure in Canadian polar bears. Molecular Ecology **4:**347–354.

Roy, M. S., E. Geffen, D. Smith, E. A. Ostrander, and R. K. Wayne. 1994. Patterns of differentiation and hybridization in North American wolflike canids, revealed by analysis of microsatellite loci. Molecular Biology and Evolution **11:**553–570.

Savolainen, O., and P. Hedrick. 1995. Heterozygosity and fitness: no association in scots pine. Genetics **140:**755–766.

Shaklee, J. B., and N. V. Varnavaskaya. 1994. Electrophoretic characterization of odd-year pink salmon populations from the Pacific coast of Russia, and comparison with selected North American populations. Canadian Journal of Fisheries and Aquatic Science **51**(Supplement 1):158–171.

Shriver, M. D., L. Jinn, R. Chakraborty, and I. Boerwinkle. 1993. VNTR allele frequency distributions under the stepwise mutation model—a computer simulation approach. Genetics **134:**983–993.

St. Louis, V. L., and J. C. Barlow. 1988. Genetic differentiation among ancestral and introduced populations of the Eurasian Tree Sparrow. Evolution **42:**266–276.

Taylor, A. C., W. B. Sherwin, and R. K. Wayne. 1994. Genetic variation of microsatellite loci in a bottlenecked species: the hairy-nosed wombat (*Lasiorhinus krefftii*). Molecular Ecology **3:**277–290.

Valdes, A. M., M. Slatkin, and N. B. Freimer. 1993. Allele frequencies at microsatellite loci—the stepwise mutation model revisited. Genetics **133:**737–749.

Varnavskaya, N. V., C. C. Wood, and R. J. Everett. 1994. Genetic variation in sockeye salmon (*Oncorhynchus nerka*) populations of Asia and North America. Canadian Journal of Fisheries and Aquatic Science **51**(Supplement 1):132–145.

Wada, S., and K. Numachi. 1991. Allozyme analyses of genetic differentiation among the populations and species of the Balaenoptera. Report of the International Whaling Commission (special issue) **13:** 126–154.

Waits, L. P. 1996. A comprehensive molecular study of the evolution and genetic variation of bears. Ph.D. dissertation. University of Utah, Salt Lake City.

Watterson, G. A. 1984. Allele frequencies after a bottleneck. Theoretical Population Biology **26:**387–407.

Winans, G. A. 1980. Geographic variation in the milkfish *Chanos chanos*. I. Biochemical evidence. Evolution **34:**558–574.

Winans, G. A., P. B. Aebersold, S. Urawa, and N. Varnavskaya. 1994. Determining continent of origin of chum salmon using genetic stock identification techniques: status of allozyme baseline in Asia. Canadian Journal of Fisheries and Aquatic Science **51**(Supplement 1):95–113.

Appendix 1

Sign tests for heterozygosity excess in 11 microsatellte data sets (first 11 listed) and 10 alloyzme data sets form populations thought to have been recently bottlenecked, based on data from demographic, biogeographic, or independent molecular studies.

Species & population[a]	*Mean no. of individuals sampled per locus*	*Sign test*[b]				*Historical population census size/date*
		SMM		*IAM*		
		H_e/H_d	p	H_e/H_d	p	
Mountain sheep (*Ovis canadensis*)						
Wildhorse Is.	25	6/0	0.04[c]	6/0	0.03[c]	8 founders/1947; 90/1954; 309/1979; 200/1994
Bison Range	23	7/0	0.009[c]	7/0	0.003[c]	12 founders/1921; 90/1929; 8/1939; 12/1949; 50/1984
Tarryall	24	5/1	0.18	6/0	0.029[c]	900/pre-1952; 44/1953; 100/1970; 200/1988
Soay sheep (*Ovis aries*)						
Herta Island	>900	6/0	0.026[c]	6/0	0.011[c]	107 founders/1932; size fluctuates between 600 and 1500 every 3–5 years
Wombats (*Lasiorhinus latifrons*)						
Epping Forest	43	8/1	0.026[c]	8/1	0.011[c]	20–30/1981; 70/1994
Brown bears (*Ursus arctos*)						
Kodiak	32	4/1	0.27	4/1	0.18	Isolated for approx. 10,000 years; low allozyme and mtDNA variation
Yellowstone	53	6/2	0.22	7/1	0.045[c]	Isolated since late 1800s; <150/1960s; >200/1990s
Koalas (*Phascolarctos cinereus*)						
Kangaroo Island	12	4/0	0.065	4/0	0.042[c]	18 founders/1924 (from the French Island population, founded with as few as 2–3 individuals in the 1880s)
Red foxes (*Vulpes vulpes*)						
San Remo	22	7/0	0.016[c]	7/0	0.014[c]	5 founders/1870, but other undocumented introductions may have occurred
Phillip Island	23	6/1	0.079	6/1	0.043[c]	Unknown number of founders from the Australian mainland
Wolves (*Canis lupus*)						
Mexican-certified	21	7/3	0.186	7/3	0.131	3 founders/1984 (from a remnant population in Mexico)
Soay sheep						
Herta Island	>900	5/0	0.015[c]	5/0	0.009[c]	listed above
Brown bears						
Western Carpathians	57	5/0	0045[c]	5/0	0.027[c]	40/1932; 700/1995; isolated since the late 1800s
Eurasian Tree Sparrow (*Passer montanus*)						
Illinois-WOOD	24	6/2	0.15	6/2	0.097	20 founders/1870
Illinois-NAPL	17	6/2	0.21	7/0	0.002[c]	20 founders/1870
Common Myna (*Acridotheres Tristis*)						
Oahu, Hawaii	38	7/0	0.006[c]	7/0	0.001[c]	About 100 founders/1882
Sidney	42	5/4	0.417	6/3	0.125	About 100 founders/1862
Galaxid fish (*Galaxias truttaceus*)						
Isabella Lagoon	40	5/0	0.03[c]	5/0	0.02[c]	Bottleneck inferred from mtDNA; population became isolated 3000–7000 years ago
Land snail (*Thebia pisana*)						
Mainland-city	27	5/0	0.022[c]	5/0	0.013[c]	Unknown number of founders/1890s
Mainland-cott	27	3/3	0.64	4/2	0.23	Unknown number of founders/1890s
One-horned rhinoceros (*Rhinoceros unicornis*)						
Nepal	22	6/4	0.367	6/4	0.279	>1000/1950; 60–80/1962; >250/1988

[a] *Wildhorse Island, Tarryall, Sun River, and Vaseux Lake: Luikart (1997); Bison Range and Sheep River: S. Forbes and J. Hog (unpublished data); Herta Island: Ban Croft et al. (1995); Epping Forest and Brookfield: Taylor et al. (1994); Kodiak, Yellowstone, Kluane, East Slope N. Continental Divide Ecosystem: Waits (1996); Kangaroo Island: Houblen et al (1996); San Remo and Phillip Island: Lade et al. (1996); Mexican certified: Garcia-Moreno et al (1996) Western Carpathians and Brooks Range: K. Knudsen, et al. (unpublished data); Illinois-Wood and NAPL, Germany, and Sweden: St. Louis and Barlow (1988); Oahu, Sidney, Bopbal, and Bhubaneswar: Baker and Moeed (1987); Isabella Lagoon, Allens Creek and Fortegue Lagoon: Ovenden and White (1989); Mainland-City and -Cott, Vale, and Centre National de la Research Scientific (CNRS): Johnson (1988); Nepal: Dinerstein and McCracken (1990).*

[b] *All data sets have at least five polymorphic loci (except the data from the severely bottlenecked koalas from Kangaroo Island). Data sets with few individuals or with individuals poled from more than one population were not tested (e.g., cheetahs; Menotti-Raymond & O'Brien 1995). Some data sets are representatives of numerous published data sets from populations of a given species (e.g., Common Myna).* H_e/H_d *represents the ratio of the number of loci with a heterozygosity excess to the number with a heterozygosity deficiency. The* H_e/H_d *ratio is expected to be approximately 1:1 for nonbottlenecked populations.* H_e *is expected to be larger than* H_d *for recently bottlenecked populations.*

[c] *Significant deviation* ($p < 0.05$) *from equilibrium (nonbottleneck) expectations.*

Appendix 2

Sign tests for heterozygosity excess in 26 microsatellite data sets from populations thought not to have been recently bottlenecked.

Species & population[a]	*Mean number of individuals sampled per locus*	*Sign test*[b]			
		SMM		*IAM*	
		H_e/H_d	p	H_e/H_d	p
Mountain Sheep					
Sun River	32	3/3	0.55	5/1	0.12
Sheep River	50	5/3	0.57	6/2	0.06
Vaseux Lake	25	2/4	0.19	2/4	0.22
Wolves					
Hinton	32	4/6	0.57	9/1	0.026[c]
Northwest Territory	21	7/3	0.34	9/1	0.033[c]
Coyotes (*Canis latrans*)					
California	22	5/5	0.40	8/2	0.115
Brown bears					
W. Brooks Range	152	4/4	0.56	7/1	0.022[c]
Kluane	51	3/5	0.18	7/1	0.06
East Slope	33	4/4	0.43	6/2	0.29
Northern Continental Divide Ecosystem	49	1/7	0.009[d]	8/0	0.014[c]
Polar bears (*Ursus meritimus*)					
W. Hudson Bay	30	3/5	0.18	5/3	0.57
Davis Strait	26	3/5	0.18	5/3	0.57
N. Beaufort	30	3/5	0.17	5/5	0.57
S. Beaufort	22	4/4	0.40	6/2	0.29
Wombats (*lasiorhinus krefftii*)					
Brookfield	16	10/4	0.23	13/1	0.00[c]
Field mice (*Mus musculus and M. domesticus*)					
M. domesticus-3	24	6/0	0.04[c]	6/0	0.02[c]
M. musculus-7.92	24	3/2	0.68	3/2	0.64
Humans (*Homo sapiens*)					
Sardinia	46	0/10	0.000[d]	0/10	0.000[d]
Egypt	46	2/8	0.052	1/9	0.013[d]
Kachari	40	5/1	0.216	5/1	0.043
New Guinea	39	3/3	0.475	4/2	0.465
Grey Seals (*Halichoerus grypus*)					
Isle of May (adults)	35	2/6	0.17	7/1	0.10
North Rona (adults)	176	3/5	0.19	7/1	0.10
Fruit flies (*Drosophila melanogaster*)					
Tyrell	68	1/7	0.01[d]	5/3	0.49
Bumble bees (*Bombus terrestris*)					
Corsica	21	3/4	0.39	5/2	0.32
Sardinia	22	2/5	0.12	3/4	0.38

[a] *Hinton: Forbes and Boyd (1996); Northwest Territory and California: Roy et al. (1994); W. Brooks Range: Craighead (1994); W. Hudson Bay, Davis Strait, N. and S. Beaufort: Paetkau et al. (1995);* M. domesticus *and* musculus*: Dallas et al. (1995); Sardinia and Egypt: DiRienzo et al. (1995); Kachari and New Guinea: Deka et al. (1991); Isle of May and North Rona: Allen et al. 1996; Tyrell: England et al. (1996); Corsica and Sardinia; Estoup et al. (1996). If location lacks a reference see Appendix 1, footnote* a.

[b] *These data sets include at least 20 individuals (except for the Brookfield wombat population) and five polymorphic loci. We attempted to use populations from relatively undisturbed habitats, such as bears* (Ursus *sp.), mountain sheep* (Ovis canadensis)*, and wolves* (Canis lupus) *from northern Alaska and Canada.* H_e/H_d *represents the ratio of the number of loci with a heterozygosity excess to the number with a heterozygosity deficiency, as in Appendix 1. Nonbottlenecked populations should have a* H_e/H_d *ratio of approximately 1:1.*

[c] *Significant deviation* ($p < 0.05$) *from equilibrium/nonbottleneck expectations.*

[d] *Significant deficiency of heterozygosity, possibly caused by recent population expansion or introduction of unique or rare alleles by immigrants.*

Appendix 3

Sign tests for heterozygosity excess in 46 allozyme data sets from populations thought not to have been recently bottlenecked.

Species & population[a]	*Mean number of individuals sampled per locus*	*Sign test*[b]			
		SMM		*IAM*	
		H_e/H_d	p	H_e/H_d	p
Mountain sheep					
Sun River	29	3/2	0.44	4/1	0.10
Wolves					
Tuktoyaktuk	93	3/2	0.46	3/2	0.31
Brown bear					
Western Brooks Range	42	2/3	0.57	2/3	0.62
Common Myna					
Bophal	40	3/12	0.02[c]	3/12	0.04[c]
Loknow	40	3/13	0.02[c]	4/12	0.10
Bhubaneswar	36	3/12	0.01[c]	4/11	0.11
Eurasian Tree Sparrows					
Germany	30	7/5	0.35	8/4	0.11
Sweden	25	5/5	0.61	6/4	0.29
Minke whales (*Balenoptera acutorostrata*)					
MKC	45	4/5	0.60	4/5	0.55
MBC	190	2/10	0.017[c]	3/9	0.12
Bryd whales (*Balenoptera edeni*)					
BMA	100	2/4	0.40	3/3	0.46
BJA	118	1/5	0.12	2/4	0.54
Galaxid fish					
Allens Creek	40	3/9	0.047[c]	3/9	0.10
Fortesue Lagoon	42	3/7	0.14	3/8	0.15
Pink salmon (*Oncorhynchus gorbuscha*)					
Ivashka	75	2/19	0.000[c]	3/18	0.00[c]
Kik-chik	78	3/13	0.009[c]	4/12	0.08
Pymta	79	4/19	0.004[c]	6/17	0.09
Arman	79	3/18	0.001[c]	5/16	0.05
Chum salmon (*Oncorhynchus keta*)					
Anadyr	100	9/17	0.14	11/15	0.46
Ola	80	11/18	0.28	12/17	0.53
Kamchatka-b	39	7/14	0.13	8/13	0.29
Sockeye salmon (*Oncorhynchus nerka*)					
Skilak	50	3/10	0.07	3/10	0.14
Yenta	50	3/4	0.52	4/3	0.34
Dalnee 89–90	250	1/6	0.12	3/4	0.54
Atlantic cod (*Gadus morhua*)					
Cod-E	95	3/5	0.37	3/5	0.53
Cod-F	96	1/6	0.06	3/4	0.64
Cod-I	98	2/3	0.49	3/2	0.36
Cod-G	96	1/6	0.06	3/4	0.64
Crabs (*Halice tridans* and *Chiromantes dehaani*)					
H. tridans-2	39	0/6	0.02[c]	0/6	0.03[c]
H. tridans-3	40	0/7	0.01[c]	0/7	0.02[c]
C. dehaani-2	39	1/7	0.04[c]	1/7	0.08
C. dehaani-1	23	1/4	0.16	1/4	0.22
Land snails					
Vale, Fance	28	4/7	0.18	6/5	0.51
CNRS, France	28	4/7	0.35	4/7	0.45
American oysters (*Crassostrea virginica*)					
Cape Cod, MA	90	0/5	0.010[c]	1/4	0.10
Charleston, SC	100	1/4	0.10	3/2	0.60
Bay Grabe, LA	88	0/5	0.011[c]	4/1	0.28
Brownsville, TX	97	1/4	0.10	5/0	0.053
Milk fish (*Chanos chanos*)					
Oahu	60	4/3	0.45	4/3	0.38
Tarawa	38	3/7	0.18	4/6	0.50
Christmas Island	47	3/3	0.62	1/5	0.09

Appendix 3
Continued

Species & population[a]	*Mean number of individuals sampled per locus*	*Sign test*[b]			
		SMM		*IAM*	
		H_e/H_d	p	H_e/H_d	p
New Zealand conifers (*Halocarpus bidwillii*)					
Pop-14	38	0/7	0.01[c]	0/7	0.03[c]
Pop-16	76	0/5	0.046[c]	1/4	0.36
Pop-2	40	0/5	0.042[c]	0/5	0.07
Pop-6	40	0/5	0.044[c]	0/5	0.07
Scots pine (*Pinus Sylvestris*)					
Yllastunturi	44	7/5	0.31	8/4	0.08

[a] *Sun River: K. Knudsen and F.W. Allendorf (unpublished data); Tuktoyaktuk: Kennedy et al. (1991); BMA and BJA: Wada and Numachi (1991); Ivashka, Kik-chik, Pymta, and Arman: Shaklee and Varnavaskya (1994); Anadyr, Ola, and Kamchatka-b: Winans et al. (1994); Skilak and Yenta: F.W. Allendorf et al. (unpublished data); Dalnee: Varnavaskaya et al. (1994); Cod-E, -F, -I, and -G: Mork et al. (1985);* H. tridans *and* C. dehaani*: Irawan et al. (1993); Cape Cod, Charleston, Bay Grabe, and Brownsville: Buroke (1983) (we used only the five loci used by Karl and Avise [1992] and suspected by them to be under balancing selection); Oahu, Tarawa and Christmas Island: Winans (1980); pop-14, -16, -2, and -6: Billington (1991); Yllastuntur: Savolainen and Hedrick (1995). If location lacks a reference, see Appendix 1, footnote a.*

[b] *These data sets include at least 20 individuals and five polymorphic loci. We attempted to use populations for relatively undisturbed habitats (e.g., Pacific salmon [*Oncorhynchus sp.*]) from remote areas without hatchery influences.* H_e/H_d *represents the ratio of the number of loci with a heterozygosity excess over the number with a heterozygosity deficiency, as in Appendices 1 and 2.*

[c] *Significant* ($p < 0.05$) *deficiency of heterozygosity, possibly caused by a recent population expansion or introduction of unique or rare alleles by immigrants.*