

Founder effect and bottleneck signatures in an introduced, insular population of elk

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Abstract The population of elk (*Cervus elaphus roosevelti*) inhabiting Afognak Island, Alaska, USA arose from an introduction of 8 individuals from an established population in Washington, USA in 1929, and recently peaked at approximately 1,400 individuals. We examined indices of diversity for 15 microsatellite loci in the Afognak population and compared them to levels in the parent population to determine effects of translocation and demography on genetic variation. The Afognak population differed significantly ($P < 0.0001$) from the source population in both allele and genotype frequencies. Allelic richness, number of private alleles and multilocus heterozygosity, but not percent loci polymorphic, were significantly lower in Afognak elk. Mean inbreeding coefficients within Afognak ($f = 0.019$) and source ($f = -0.006$) populations did not differ significantly from zero. Despite the demographic bottleneck, no evidence of a genetic bottleneck in the Afognak population was detected using a test for heterozygosity excess or mode shift of allele frequencies. Simulations indicated that rapid population growth after the translocation resulted in heterozygosity excess for only 8 years. Conversely, a statistic testing for a bottleneck signature in the ratio of allele number to allele size range (M -ratio) was significant for both the Afognak and source populations, suggesting that the Afognak population had effectively undergone serial bottlenecks. Nonetheless, Afognak failed to show a smaller M -ratio than

the parent population, suggesting a failure of that statistic to detect the bottleneck associated with introduction. We show that a severe bottleneck followed by rapid population growth may be undetectable using available tests.

Keywords *Cervus elaphus* · Genetic diversity · Heterozygosity excess · M -ratio · Microsatellites · Wildlife introductions

Introduction

There exist few studies of effects of severe reduction in size of wild populations of vertebrates wherein data concerning initial bottleneck size, genetic characteristics of pre- and post-bottleneck populations, and population growth are known, and the response of the introduced population is not confounded by immigration. Consequently, case studies of such populations can yield valuable insight into dynamics of genetic markers under constraints of demographic bottlenecks and subsequent changes in population size. Those instances also provide opportunities for evaluating statistical tests of bottlenecks.

A genetic bottleneck causes loss of allelic diversity and heterozygosity (Nei et al. 1975; Allendorf 1986). Rare alleles are most likely to be lost (Nei et al. 1975; Houlden et al. 1996; Luikart et al. 1998; England et al. 2003), which can be visualized as a departure from an L-shaped distribution of allele frequencies (Luikart et al. 1998). Moreover, loss of rare alleles causes a temporary excess in heterozygosity because heterozygosity expected for a given number of alleles at mutation-drift equilibrium (H_{eq}) is affected more by loss of rare alleles than is heterozygosity expected under Hardy–Weinberg (H_E), which forms a basis

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for detection of bottlenecks (Cornuet and Luikart 1996). A second method of testing for bottlenecks operates under the principle that a significant bottleneck will cause a greater proportional loss of alleles than a proportional loss of range of allele size and the ratio of those parameters (M -ratio) will be lower for bottlenecked populations. It has been successful in detecting bottlenecks in a variety of taxa (Garza and Williamson 2001). Both methods were found to have low Type I and Type II error rates compared with other methods (Williamson-Natesan 2005).

We used an isolated, translocated population of Roosevelt elk (*Cervus elaphus roosevelti*) to investigate the genetic response of a population to founder effect without subsequent gene flow and to test popular methods for detecting bottlenecks. Five female and three male Roosevelt elk were introduced to the Kodiak Archipelago, specifically Afognak and Raspberry Islands, Alaska, USA (Fig. 1) in March 1929. All of those animals had been collected as calves in 1928 from the Hoh River Valley, Olympic Peninsula, Washington, USA and had been shipped to Alaska at 3 months of age where they were raised in captivity until their release (Burris and McKnight 1973). The age at which the animals were transported to Alaska ensured that none of the females was pregnant by a non-translocated male at release. The fates and reproductive histories of individual animals after the introduction are unknown, so the actual size of the founding population may have been smaller than 8. There have been no

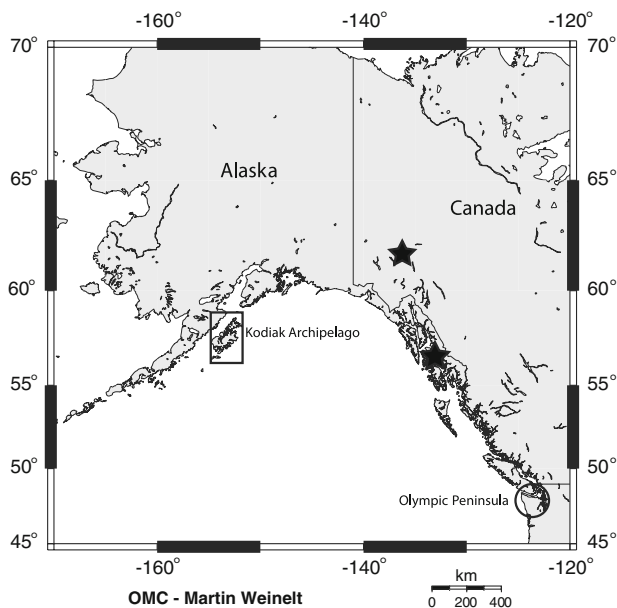


Fig. 1 Map of Pacific Northwest indicating the locations of the Olympic Peninsula, Washington (circle), Kodiak Archipelago, Alaska (rectangle), containing Afognak and Raspberry Islands, and the two wild populations of elk in North America nearest to the Afognak population (stars)



Fig. 2 Reconstruction of population size of Roosevelt elk on Afognak Island since introduction, based on unpublished local records

additional introductions; moreover, the archipelago is more than 1,000 km from the nearest population of wild elk and isolation of the Kodiak Archipelago makes immigration virtually impossible (Fig. 1). The elk population on Afognak and Raspberry Islands has thrived, with a maximum population size of approximately 1,400 animals. Rapid population growth in the 40 years after founding was followed by a severe population reduction ($\sim 70\%$) in the early 1970s due to a succession of severe winters. Population size returned to pre-reduction levels and was considered to be stable at the time of this study (Fig. 2).

We compared observed genetic variation in the introduced elk population with that of the source population and theoretical expectations, particularly with reference to testing for historical bottlenecks. We hypothesized that Afognak elk would show reduced genetic diversity proportionate to the bottleneck size and that signatures of a bottleneck would be detectable by popular test methods.

Materials and methods

To assess genetic variation in elk, we examined microsatellites because of their high degree of polymorphism. Tissue samples were solicited from hunters and biologists and were stored frozen until analyzed; samples consisted of muscle, skin or plucked hair. We analyzed 34 samples from Afognak and Raspberry elk (hereafter referred to as Afognak) and 22 from the Olympic Peninsula (Olympic). A commercial laboratory (Wildlife Genetics International, Nelson, British Columbia, Canada) conducted DNA extraction, amplification and genotyping. Dinucleotide microsatellite loci BL42, BM203, BM888, BM4028, BM4107, BM4513, BM6506, BMC1009 (Bishop et al. 1994), OarFCB193 (Buchanan and Crawford 1993), CSSM041 (Moore et al. 1994), INRA107 (Vaiman et al. 1994), ODOSTSH (referred to as Cervid14 in DeWoody et al. 1995), Rt1, Rt7, and Rt13 (Wilson et al.

1997) were selected to characterize the elk genome. Amplification of all loci followed standard protocols described in those publications with slight modifications to facilitate multiplexing. That suite of markers was chosen for a high degree of polymorphism and because they could be multiplexed in a single lane on an automated sequencer.

We computed observed heterozygosity (H_O) and H_E from allele frequencies. Allelic richness (alleles per locus, A) for a given sample size was estimated by counting alleles and by using rarefaction (Kalinowski 2005) to account for differences in sample sizes between populations. We measured allele and genotype frequencies of each population and tested for differences between populations and for deviations from Hardy-Weinberg expectations within populations using software GENEPOP (Raymond and Rousset 1995). Rarefaction estimates of allelic richness and unique alleles per locus were estimated using HP-RARE (Kalinowski 2005) and differences between populations were tested using a sign test. Linkage disequilibrium was measured by Fisher's exact test for two-locus associations and was adjusted for within-locus disequilibrium; we conducted that analysis with GENEPOP. Disequilibrium across all loci was determined by applying Fisher's method for combining P -values. Inbreeding coefficients for animals within populations (f) and an index of population differentiation (F_{ST}) were calculated from variance in allele frequencies using the method of Weir and Cockerham (1984) as implemented by software GDA (Lewis and Zaykin 2002). Estimates of 95% confidence intervals around inbreeding estimates were generated using 10,000 bootstrap replicates of data.

Evidence for a genetic bottleneck was evaluated using software BOTTLENECK (Piry et al. 1999), which assumed that a signature of a severe reduction in effective size of a population was an excess of H_E relative to H_{eq} (Cornuet and Luikart 1996). We tested for such an excess using a Wilcoxon signed-rank test (Cornuet and Luikart 1996) under a two-phase model (TPM) of microsatellite evolution (Di Rienzo et al. 1994). We constrained the model by defining 90% of mutations as conforming to a stepwise mutation model (SMM; Kimura and Ohta 1978) and 10% as multi-step, and assuming a variance (σ_g^2) of 12 for the geometric distribution of number of repeat units per multi-step mutation. Mean step size for multi-step mutations (Δ_g) is roughly equal to σ_g (Di Rienzo et al. 1994), which equates in our analysis to 3.5 repeat units. That parameterization of the TPM was recommended as a conservative model of microsatellite mutation (Garza and Williamson 2001). We also conducted an evaluation of the distribution of allele frequencies in the Afognak population to determine if a mode shift characteristic of a bottleneck had occurred (Luikart et al. 1998) as determined by BOTTLENECK.

We conducted a second test for a bottleneck by examining the M -ratio (Garza and Williamson 2001). Loci were excluded from analysis that were monomorphic or that contained an allele size not representing an integer multiple of the repeat unit size (Garza and Williamson 2001). We generated a critical value (M_c) at $\alpha = 0.05$ by which to test estimates of M for evidence of a bottleneck using software Critical_M.exe (available from the website <http://swfsc.noaa.gov/textblock.aspx?Division=FED&id=3298>) that incorporated sample size (individuals and loci) in 10,000 permutations of data employing the same conservative parameterization of the TPM as the test for heterozygosity excess with the additional specification of $\theta (= 4N_e\mu) = 1$ (Garza and Williamson 2001).

We computed expected initial heterozygosity (H_E) of the Afognak founders by using the equation

$$H_E = H_O(1 - 1/2N),$$

where N is the number of founders and H_O is heterozygosity of the population from which the founders were selected, which we assumed was equal to heterozygosity of the current Olympic population. We compared observed difference in number of alleles between populations with the expected difference from a founding event using the equation

$$a = n - \sum (1 - p_i)^{2N},$$

where a is expected number of alleles at a locus in the founded population, n is number of alleles at that locus in the source population, p_i is frequency of the i th allele in the source population, and N is the size of the founded population.

We compared genetic characteristics of the Afognak population with those of simulated populations using software BOTTLESIM (Kuo and Janzen 2003). We ran the model for 1,000 iterations with non-constant population size, random mating, generations overlapping by 80%, reproductive maturity at 1 year of age, age of senescence at 15 years, and 60% of the population as females. We constructed an initial population with the genetic characteristics of the Olympic population and subjected it to a bottleneck of 8 animals (5 females). A population reconstruction of the Afognak population (Fig. 2), based on local records, was used in the simulations as a model of temporal variation in post-introduction population size. That reconstruction approximated a population rate of increase of 14.6%, which is certainly within the realm of possibility for a newly introduced population of elk (Eberhardt et al. 1996). The software provided means for H_E , and A for the initial population and each of 70 years of simulation. We used locus-specific means of A , rounded to the nearest integer, and H_E from specific years of the simulation as

input for BOTTLENECK to determine temporal trends in heterozygosity excess.

Results

Genetic diversity

All loci analyzed in this study were polymorphic in at least one population (Table 1); only locus Rt13 in Afognak elk exhibited a single allele. Neither population deviated from Hardy–Weinberg expectations (Afognak: $\chi^2 = 23.7$, $P = 0.59$; Olympic: $\chi^2 = 31.4$, $P = 0.40$) but exact tests revealed 1 locus in Afognak elk (OarFCB193: $P = 0.024$) and 1 in Olympic elk (BM6506: $P = 0.015$) that deviated significantly from equilibrium. Two significant deviations out of 30 comparisons is within the realm of chance at $\alpha = 0.05$; therefore, all loci were included in further analysis. For all loci, no linkage disequilibrium was detected in either population (Afognak: $\chi^2 = 225.9$, $P = 0.21$; Olympic: $\chi^2 = 147.6$, $P = 0.99$). Nonetheless, the number of instances of pairwise disequilibria in Afognak (9) was greater than the number observed in Olympic (1) as determined by Fisher's exact test ($P = 0.0093$).

Elk from Afognak differed from Olympic elk in both allele and genotype frequencies. Allele frequencies differed at 10 of 15 loci and genotype frequencies differed at 11 of 15 loci. Despite observed differences in allele frequencies, there was a significant correlation of frequencies of alleles between populations ($r = 0.84$, $P < 0.001$) and F_{ST} was neither exceedingly high nor low (0.083). All alleles absent in Afognak were uncommon (present at frequency < 0.1) in Olympic. Three of 18 uncommon alleles in Olympic were retained in the introduced population. Of 3 alleles absent from Olympic, all were uncommon in Afognak.

Individual elk from Olympic exhibited greater multilocus heterozygosity than those from Afognak ($t = -3.5$, $P_{(1\text{-tailed})} = 0.0004$; Fig. 3). Genetic indices for Afognak elk, with the exceptions of percent loci polymorphic and inbreeding coefficient, differed significantly from those of Olympic elk and showed effects of founding (Table 2). Estimates of f for each population were low and 95% confidence intervals encompassed zero for both populations (Table 2); thus, we were unable to reject a null hypothesis of no inbreeding in Afognak. Private alleles were detected at 11 of 15 loci; Olympic elk had $5\times$ as many private alleles as Afognak elk despite a smaller sample size (Table 2). Standardizing mean private alleles per locus using rarefaction resulted in an even greater disparity between populations (Table 2). Rarefaction

Table 1 Alleles detected at 15 microsatellite loci in Roosevelt elk from Afognak Island, Alaska, and Olympic Peninsula, Washington

Locus	Allele size	Afognak	Olympic
BL42	250	0.074	0.182
	258	0.794	0.500
	260	0.132	0.318
BM203	223	0.044	0.114
	225	0.000	0.068
	226	0.250	0.068
	228	0.088	0.182
	229	0.603	0.386
BM888	231	0.015	0.182
	180	0.044	0.318
	188	0.662	0.545
	190	0.294	0.136
BM4028	109	0.985	0.955
	111	0.015	0.000
	121	0.000	0.023
BM4107	123	0.000	0.023
	157	0.574	0.523
	167	0.000	0.045
BM4513	169	0.426	0.432
	122	0.000	0.023
	124	0.691	0.432
BM6506	130	0.309	0.545
	207	0.652	0.227
	209	0.045	0.205
	211	0.000	0.045
	215	0.197	0.409
BMC1009	217	0.106	0.114
	283	0.647	0.659
	285	0.353	0.318
CSSM041	287	0.000	0.023
	125	0.529	0.738
	129	0.000	0.071
OarFCB193	131	0.471	0.190
	97	0.324	0.136
	119	0.015	0.000
	121	0.191	0.432
	123	0.000	0.023
	137	0.000	0.045
	139	0.000	0.023
	141	0.426	0.045
INRA107	143	0.000	0.023
	145	0.044	0.273
	158	0.136	0.227
	160	0.621	0.477
	170	0.000	0.091
	172	0.242	0.205

Table 1 continued

Locus	Allele size	Afognak	Olympic
ODOSTSH	133	0.221	0.214
	135	0.647	0.571
	137	0.118	0.119
	139	0.015	0.000
	143	0.000	0.095
Rt1	217	0.955	0.786
	229	0.045	0.214
Rt7	224	0.364	0.619
	226	0.045	0.143
	230	0.576	0.190
	232	0.015	0.048
Rt13	297	0.000	0.075
	305	1.000	0.925

estimates were based on a sample size of 19 because that was the number of animals in Olympic for which we had complete genotypes. All private alleles were uncommon. Neither population exhibited a deficit of uncommon alleles and no mode shift was detected (data not shown). Mean (SE) frequency of occurrence for private alleles in Afognak was 0.015 (0) whereas that for Olympic was 0.048 (0.016), a difference that was significant (t for arcsine-transformed data = -5.8 , $P < 0.0001$).

Expected heterozygosity among a random selection of 8 individuals from Olympic, representing the founder population, was 0.49, which was greater than the current estimate of 0.42 that we estimated from allele frequencies for Afognak (Table 2). Assuming that all observed alleles were present in the original source population (including alleles observed in Afognak but not Olympic in this study), we would expect a total reduction of allele number from 63 in the source population to 48.5 in the founded population

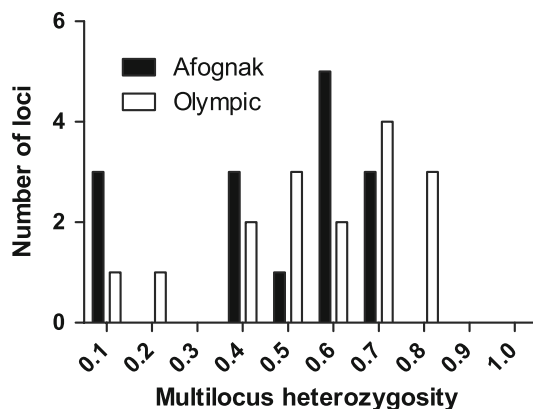


Fig. 3 Distributions of individual multilocus heterozygosity for 15 microsatellite loci in Afognak and Olympic elk populations

when summed across all loci; we observed 48 alleles in Afognak elk.

Bottleneck detection

We detected two loci in Afognak and one in Olympic that differed significantly from expectations of heterozygosity under mutation-drift equilibrium conditions. Loci CSSM041 and BM4107 exhibited excess heterozygosity in Afognak whereas locus BM4028 showed a deficit in Olympic. On a population basis, mean H_{eq} , calculated as the unweighted mean of locus-specific estimates of H_{eq} from BOTTLENECK, was 0.41 for Afognak and was 0.52 for Olympic. We observed no significant excess of heterozygosity across all loci in either population (Afognak: $P = 0.31$; Olympic: $P = 0.24$) and therefore were unable to reject the null hypothesis of no bottleneck.

For estimation of M , we eliminated locus Rt13 from Afognak because it was monomorphic and locus BM203 from both populations because it contained allele sizes not representing addition or subtraction of complete repeat units (Table 1). Mean M for Afognak (0.57) was marginally greater than mean M for Olympic (0.56), which was unexpected if Olympic was a stable population with large N_e and Afognak was bottlenecked. The 95% threshold value, M_c , was 0.79, indicating that both populations exhibited evidence of a reduction of N_e . Nonetheless, considering that M for Afognak was not indicative of a more severe bottleneck than Olympic it may be that M failed to detect the bottleneck associated with introduction.

Simulations

Simulations began with $H_E = 0.52$ in the source population at year 0 (prior to introduction), with initial allele frequencies adjusted to include all alleles detected in this study, including those found only in Afognak. After reduction of population size to 8 elk (5 females) to simulate founding of the Afognak population, mean (SE) alleles per locus declined immediately by 19%, from 3.9 (0.46) to 3.1 (0.28), and thereafter became stable at approximately 2.97 (0.26; Fig. 4), similar to our estimate for Afognak elk (Table 2). Estimates of multilocus H_{eq} exhibited the same pattern as alleles per locus, which is to be expected because H_{eq} is estimated from allele number (Luikart et al. 1998). The sharp declines in A and H_{eq} immediately after introduction were not seen in H_E , which declined by 7% in the first year and then declined more slowly over a period of approximately 10 years to 0.458 (0.045; Fig. 4) and was 0.452 (0.045) in year 70. Overall, A was reduced by 24% and H_E was reduced by 12% by year 10. Significant heterozygosity excess was observed in years 1-8 of the

Table 2 Indices of genetic diversity for populations of elk (*C. elaphus roosevelti*) from Olympic Peninsula, Washington and Afognak Island, Alaska

Metric	Afognak ($n = 34$)	Olympic ($n = 22$)	Significance level for population difference (P for 1-tailed test)
Percent loci polymorphic (P)	93	100	0.500 ^b
Allelic richness	2.9	3.7	Not tested
Allelic richness (A_{19}) ^a	2.76	3.67	<0.0001 ^c
Private alleles (A_P)	3	15	<0.0001 ^b
Private alleles per locus ^a	0.11	1.02	<0.0001 ^c
Expected heterozygosity (H_E)	0.42	0.53	0.0003 ^d
Observed heterozygosity (H_O)	0.41	0.53	0.0015 ^d
Inbreeding coefficient (f) (95% CI)	0.019 (−0.063–0.102)	−0.006 (−0.10–0.090)	0.37 ^d

Tests of differences between populations are 1-tailed, corresponding to hypotheses that the Afognak population would show lesser (P , A , A_P , H_E , H_O) or greater (f) values than the Olympic population due to founder effect. Uncorrected values for allelic diversity were not tested because of unequal sample sizes. Values in parentheses for the inbreeding coefficient represent a 95% confidence interval constructed from 1,000 bootstrap replicates

^a Rarefaction estimates for a sample of 19 individuals

^b Fisher's exact test

^c Sign test

^d Paired t -test

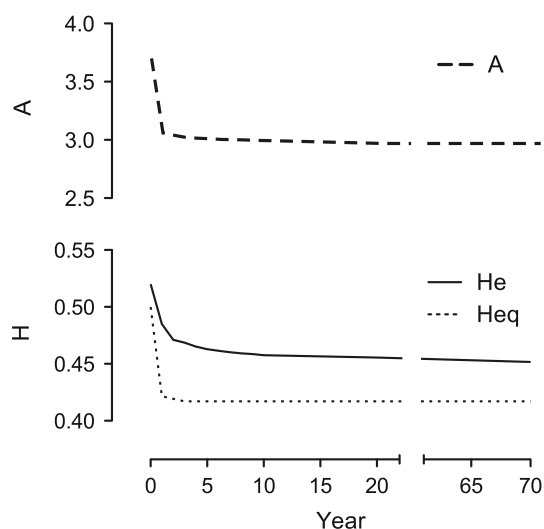


Fig. 4 Temporal changes in alleles per locus (A), expected (H_E) and equilibrium (H_{eq}) heterozygosity as predicted by a simulation of the Afognak elk population after the bottleneck. In the simulation, year 0 represents the pre-bottleneck (source) population and the bottleneck occurred in year 1. Population size changed as indicated in Fig. 2

simulations but not thereafter due to convergence of values of H_E and H_{eq} . In year 70, mean H_E generated from simulations was within 1 SE of the value of H_E estimated from our data (0.42), the simulation estimate of multilocus H_{eq} (0.417), and the estimate of multilocus H_{eq} (0.41) from our data. The decline in population size of approximately 70% in years 40–42 (Fig. 2) had no effect on genetic diversity thereafter.

Discussion

The founder effect imposed on Afognak elk due to a severe bottleneck has resulted in a significant reduction in allelic diversity and heterozygosity but not percent loci polymorphic compared with the source population from the Olympic Peninsula. Similar results were documented by Spencer et al. (2000) for experimentally created bottlenecks. Simulations documented an immediate bottleneck-induced decline in allelic richness that declined only slightly thereafter, as expected (Nei et al. 1975). In contrast, estimates of H_E declined for approximately 10 years after the bottleneck.

Despite the demonstrated effect of the bottleneck on genetic diversity in Afognak elk, we failed to detect an excess of H_E relative to H_{eq} that would be indicative of such a bottleneck. Our data revealed that alleles occurring at low frequencies (<0.1) were the most abundant frequency class of allele in both populations, despite the bottleneck. Moreover, we failed to detect a greater measure of inbreeding in the founded population compared with the source population. Any inbreeding that may have occurred after the introduction has since been eliminated through panmixia.

Testing for a bottleneck using the M -ratio produced an entirely different result, with both populations exhibiting similar ratios indicative of bottlenecks. Nonetheless, the test failed to detect a bottleneck in Afognak relative to Olympic despite the reduction in allelic diversity we documented. The only indication of a bottleneck in Afognak elk, aside

from reduced diversity, was the greater amount of linkage disequilibrium present as indicated by the exact test.

The outcome of the M -ratio tests may indicate that the test failed to detect the bottleneck of founding but rather was responding in both cases to an historical bottleneck prior to founding of Afognak. The M -ratio may be more sensitive to older bottlenecks than are tests for heterozygosity excess (Spear et al. 2006; Marshall et al. 2009). Elk populations in Olympic National Park were low enough at the turn of the 19th century that a 30-year moratorium on hunting was invoked in 1905 (Beschta and Ripple 2008). We cannot say, however, if that nadir in population size was the bottleneck for which we find evidence or if it occurred further back in history. Roosevelt elk on Vancouver Island were found to have low diversity as well (Polzhien et al. 2000), suggesting that the subspecies as a whole may have undergone a bottleneck some time in the past.

Our comparison of Afognak to its source population in Olympic National Park relies on the assumption that the population that yielded the Afognak founders was genetically identical to the one we sampled some 70 years later. The most problematic occurrence would have been a significant change in population size in Olympic between those times but evidence suggests that the Olympic population has been stable in the interim (Houston et al. 1990; Beschta and Ripple 2008).

Hidden genetic structure within a population may hinder detection of heterozygosity excess (Cornuet and Luikart 1996). Sampling that fails to account for population subdivision can yield departures from Hardy–Weinberg equilibrium due to non-random mating. Although the introduced population of elk is managed as 10 separate herds, we detected no population-level departures from Hardy–Weinberg equilibrium. Thus, we found no evidence of population subdivision and cannot reject a hypothesis of panmixia in the population.

Heterozygosity excess is detectable from approximately $0.2-4N_e$ generations after a bottleneck, with N_e being the bottleneck effective size (Luikart and Cornuet 1998). Assuming a generation length of 4 years for elk and N_e for the founded population of 7.5, the duration of the signature should be from 6 to 120 years. Results of simulations indicated that immediate, fast growth of the population minimized loss of alleles, and therefore minimized reduction of H_{eq} , negating any opportunity to detect the bottleneck more than a few years afterwards. It is often overlooked that the test for detection of heterozygosity excess assumes that the demographic bottleneck was immediate and permanent (Luikart and Cornuet 1998). For Afognak elk, the former condition was met but the latter obviously was not.

The test for heterozygosity excess may be sensitive to addition of alleles to the population (Busch et al. 2007), particularly rare alleles that do not contribute much to observed or expected heterozygosity. We have assumed that all private alleles observed in this study were present in the source population at the time that the founders were captured but it is possible that some may have arisen through mutation. Private alleles occurring at significantly lower frequencies in Afognak support the possibility of newly arisen alleles, which are more likely to be retained in a rapidly growing population than a stable one. Eliminating rare alleles from the analysis may have the potential to change results but when we eliminated the 4 private alleles from Afognak and replaced them with the most common alleles at those loci we observed no mode shift and failed to reject the hypothesis of no heterozygosity excess ($P = 0.095$). M -ratio for Afognak changed marginally from 0.57 to 0.54. Although our conclusions did not change, we submit that accumulation of new alleles through mutation must be considered as a confounding factor for testing populations many generations after the bottleneck that also underwent substantial population growth in the interim.

Empirical tests of statistical methods for bottleneck detection have been equivocal (Williamson-Natesan 2005; Marshall et al. 2009). Luikart and Cornuet (1998) evaluated the sign test (Cornuet and Luikart 1996) on microsatellite data from 11 natural populations known to have undergone bottlenecks. The test detected heterozygosity excess in only 5 populations when the SMM was assumed. The SMM is a conservative model for bottleneck testing (Luikart and Cornuet 1998) and the sign test is less powerful than the Wilcoxon test that we used (Maudet et al. 2002). A test of bottlenecked populations of alpine ibex (*Capra ibex*) in Europe using the TPM was more successful (Maudet et al. 2002). Other tests of bottlenecks have been affected by documented mutations and gene flow from adjacent populations (e.g., Busch et al. 2007). Pearce et al. (2006) found only partial correspondence between tests for heterozygosity excess and M -ratio. Spong and Hellborg (2002) reported no evidence of a genetic bottleneck for Scandinavian lynx (*Lynx lynx*) using the heterozygosity-excess test or by testing for a mode shift in allele frequencies despite a 95% reduction in lynx population size and a demographic bottleneck of as few as 30 individuals. The nadir in the lynx population occurred in the 1920s and the population reportedly numbers approximately 2000 currently, which represents a population expansion similar in magnitude and duration to that of Afognak elk.

A bottleneck was detected in an introduced herd of elk in Pennsylvania, USA despite a founding population size 4 times as large as that for Afognak. That population differed from Afognak in that it remained at a small size for

approximately 60 years after founding and only recently exhibited rapid growth (Lenney Williams et al. 2002). Population growth rate after a bottleneck is important in determining the extent of reduction in heterozygosity (Nei et al. 1975). Although heterozygosity declined during the initial growth phase of the Afognak population, the rapid rate of growth undoubtedly precluded a more significant decline.

Bottleneck detection is critical to interpretation of historical demography of populations and can be a valuable tool for endangered species management. Evidence continues to accumulate, however, that existing tests for bottlenecks do not perform well under all demographic scenarios and that possibility of Type I errors may be more likely than anticipated (Guinand and Scribner 2003; Williamson-Natesan 2005). Our data indicate that rapid growth of a population after a severe bottleneck can yield significantly reduced genetic diversity but no signature of a bottleneck within a relatively short time. That effect may be exacerbated by addition of rare alleles to the population through mutation or immigration (Busch et al. 2007). We recommend caution in implementation and interpretation of bottleneck tests when the population under consideration has had a potential for growth and suggest evaluation of multiple lines of evidence rather than relying solely on the behavior of neutral genetic markers.

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References

- Allendorf FW (1986) Genetic drift and the loss of alleles versus heterozygosity. *Zoo Biol* 5:181–190
- Beschta RL, Ripple WJ (2008) Wolves, trophic cascades, and rivers in the Olympic National Park, USA. *Ecohydrology* 1:118–130
- Bishop MD, Kappes SM, Keele JW, Stone RT, Sunden SLF, Hawkins GA et al (1994) A genetic linkage map for cattle. *Genetics* 136:619–639
- Buchanan FC, Crawford AM (1993) Ovine microsatellites at OarFCB11, OarFCB128, OarFCB193, OarFCB266 and OarFCB304 loci. *Anim Genet* 24:145
- Burris OE, McKnight DE (1973) Game transplants in Alaska. *Wildl. Tech. Bull.* 4. Alaska Department of Fish and Game, Juneau, Alaska
- Busch JD, Waser PM, DeWoody A (2007) Recent demographic bottlenecks are not accompanied by a genetic signature in banner-tailed kangaroo rats (*Dipodomys spectabilis*). *Mol Ecol* 16:2450–2463
- Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* 144:2001–2014
- DeWoody JA, Honeycutt RL, Skow LC (1995) Microsatellite markers in white-tailed deer. *J Hered* 86:317–319
- Di Rienzo A, Peterson AC, Garza JC, Valdes AM, Slatkin M, Friemer NB (1994) Mutational process of simple-sequence repeat loci in human populations. *Proc Natl Acad Sci USA* 91:3166–3170
- Eberhardt LE, Eberhardt LL, Tiller BL, Cadwell LL (1996) Growth of an isolated elk population. *J Wildl Manage* 60:369–373
- England PR, Osler GHR, Woodsworth LM, Montgomery ME, Briscoe DA, Frankham R (2003) Effects of intense versus diffuse population bottlenecks on microsatellite genetic diversity and evolutionary potential. *Conserv Genet* 4:595–604
- Garza JC, Williamson EG (2001) Detection of reduction in population size using data from microsatellite loci. *Mol Ecol* 10:305–318
- Guinand B, Scribner KT (2003) Evaluation of methodology for detection of genetic bottlenecks: inferences from temporally replicated lake trout populations. *C R Biol* 326:S61–S67
- Houlden BA, England PR, Taylor AC, Greville WD, Sherwin WB (1996) Low genetic variability of the koala *Phascolarctos cinereus* in south-eastern Australia following a severe population bottleneck. *Mol Ecol* 5:269–281
- Houston DB, Schreiner EG, Moorhead BB, Krueger KA (1990) Elk in Olympic National Park: will they persist over time? *Nat Areas J* 10(11):6–11
- Kalinowski ST (2005) HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. *Mol Ecol Notes* 5:187–189
- Kimura M, Ohta T (1978) Stepwise mutation model and distribution of allelic frequencies in a finite population. *Proc Natl Acad Sci USA* 75:2868–2872
- Kuo C-H, Janzen FJ (2003) BOTTLESIM: a bottleneck simulation program for long-lived species with overlapping generations. *Mol Ecol Notes* 3:669–673
- Lenney Williams C, Serfass TL, Cogan R, Rhodes OE Jr (2002) Microsatellite variation in the reintroduced Pennsylvania elk herd. *Mol Ecol* 11:1299–1310
- Lewis PO, Zaykin D (2002) GDA (genetic data analysis): computer program for the analysis of allelic data. Version 1.1. Available via <http://lewis.eeb.uconn.edu/lewishome/software.htm>
- Luikart G, Cornuet J-M (1998) Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conserv Biol* 12:228–237
- Luikart G, Allendorf FW, Cornuet JM, Sherwin WB (1998) Distortion of allele frequency distributions provides a test for recent population bottlenecks. *J Hered* 89:238–247
- Marshall JC, Kingsbury BA, Minchella DJ (2009) Microsatellite variation, population structure, and bottlenecks in the threatened copperbelly water snake. *Conserv Genet* 10:465–476
- Maudet C, Miller C, Bassano B, Breitenmoser-Wursten C, Gauthier D, Obexer-Ruff G et al (2002) Microsatellite DNA and recent statistical methods in wildlife conservation management: applications in Alpine ibex [*Capra ibex (ibex)*]. *Mol Ecol* 11:421–436
- Moore SS, Byrne K, Berger KT, Barendse W, McCarthy F, Womack JE et al (1994) Characterization of 65 bovine microsatellites. *Mamm Genome* 5:84–90
- Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. *Evolution* 29:1–10
- Pearce DE, Arndt AD, Valenzuela N, Miller BA, Cantarelli V, Sites JW (2006) Estimating population structure under nonequilibrium conditions in a conservation context: continent-wide population genetics of the giant Amazon river turtle. *Mol Ecol* 15:985–1006
- Piry S, Luikart G, Cornuet J-M (1999) BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. *J Hered* 90:502–503
- Polzhen RO, Hamr J, Mallory FF, Strobeck C (2000) Microsatellite analysis of North American wapiti (*Cervus elaphus*) populations. *Mol Ecol* 9:1561–1576

- Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J Hered* 86:248–249
- Spear SF, Peterson R, Matocq MD, Storfer A (2006) Molecular evidence for historical and recent population size reductions of tiger salamanders (*Ambystoma tigrinum*) in Yellowstone National Park. *Conserv Genet* 7:605–611
- Spencer CC, Neigel JE, Leberg PL (2000) Experimental evaluation of the usefulness of microsatellite DNA for detecting demographic bottlenecks. *Mol Ecol* 9:1517–1528
- Spong G, Hellborg L (2002) A near-extinction event in lynx: do microsatellites tell the tale? *Conserv Ecol* 6(1):15. [online] URL: <http://www.consecol.org/vol6/iss1/art15>
- Vaiman D, Mercier D, Moazami-Goudarzi K, Eggen A, Ciampolini R, Lepingle A et al (1994) A set of 99 cattle microsatellites: characterization, syntenic mapping, and polymorphism. *Mamm Genome* 5:288–297
- Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358–1370
- Williamson-Natesan EG (2005) Comparison of methods for detecting bottlenecks from microsatellite loci. *Conserv Genet* 6:551–562
- Wilson GA, Strobeck C, Wu L, Coffin JW (1997) Characterization of microsatellite loci in caribou (*Rangifer tarandus*), and their use in other artiodactyls. *Mol Ecol* 6:697–699