REDUCED GENETIC DIVERSITY IN TWO INTRODUCED AND ISOLATED MOOSE POPULATIONS IN ALASKA

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ABSTRACT: I examined indices of genetic diversity in 2 isolated moose (*Alces alces*) populations in Alaska that were founded by low numbers of individuals to determine effects of founding and infer whether subsequent gene flow has occurred with surrounding moose populations. Kalgin Island is a small, predator-free island in Cook Inlet that was founded by 6 moose (3 females) in the late 1950s; its population has since undergone dramatic fluctuations. Berners Bay is an isolated population along the coast of southeastern Alaska that was founded by 21 calves introduced in 1958-1960. Genetic attributes of those populations were compared to a population in Yukon Flats in central Alaska that served as an outbred control. Indices from 11 microsatellite markers indicated substantial effects of founding and subsequent isolation. Heterozygosity and allelic diversity, both of which are reduced by genetic bottlenecks, were significantly lower in the introduced populations than the Yukon Flats population. Kalgin Island diversity was significantly lower than that for Berners Bay, and was likely due to the smaller founding size and subsequent population fluctuations. Neither introduced population exhibited evidence of gene flow from surrounding populations. Managers should consider the isolation of those populations when assessing risks to population viability and crafting management strategies.

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One of the primary concerns of conservation biology is the loss of genetic diversity through genetic drift in small populations. In cases where populations are isolated, thus preventing immigration from neighboring populations, genetic drift occurs at a maximum rate depending on population size. Loss of diversity from drift is compounded in populations that are founded by low numbers of individuals due to demographic and genetic bottlenecks.

Loss of genetic diversity has been related to loss of fitness (Reed and Frankham 2003). In ungulates, studies have found correlations between indices of diversity and reduction in juvenile survival (Coulson et al. 1999, Mainguy et al. 2009, Silva et al. 2009), variation in horn/antler growth (Scribner and Smith 1990, Von Hardenberg et al. 2007), and parasite resistance (Coltman et al. 1999). Thus, genetic diversity of populations should be a primary concern in ungulate management, particularly with small and isolated populations.

Valuable insight may be gained from studying wild populations with known demographic histories to determine effects of population size on genetic diversity. Introduced populations often act as natural experiments in that regard, particularly when founding population size is known, as well as demographic trends since founding. I compared 2 small, isolated, introduced populations of moose (*Alces alces*) in Alaska to determine the effect of their respective demographic histories on indices of genetic diversity, and to infer the degree to which gene flow has affected diversity. I also compared those populations to an outbred moose population to demonstrate the extent to which the introduced populations have lost diversity.

STUDY AREA

Moose populations in Kalgin Island and Berners Bay, Alaska have similar histories. They both were established through introduction of individuals from southcentral Alaska in the late 1950s. The population on Kalgin Island (60º 27'N, 152º 00'W) was founded by 6 moose (3 females) transported to the island in 1957-1959 (Burris and McKnight 1973). The population in Berners Bay (58º 45'N, 134º 50'W) was founded by 15 calves in 1958 and 6 additional calves in 1960 (Burris and McKnight 1973). Moreover, both populations are seemingly isolated from neighboring moose populations. Kalgin Island is located in Cook Inlet which is characterized by strong tidal currents that have kept large mammals, including predators, from colonizing the island. Nonetheless, the short distance from mainland to island (<10 km) has fueled speculation that gene flow is possible. Berners Bay is separated from neighboring moose populations by rugged coastline characterized by mature spruce-hemlock (*Picea sitchensis-Tsuga heterophylla*) forest that is avoided by moose (Hundertmark et al. 1990). Predators of moose occurring in Berners Bay are wolves (*Canis lupus*), brown bears (*Ursus arctos*), and black bears (*U. americanus*).

The moose population on Kalgin Island has undergone dramatic fluctuations in population size due to density-dependent effects of habitat and periods of intense harvest, increasing in size to an estimated 212 individuals in 1982, declining to 8 in 1986, and increasing since then (Bowyer et al. 1999). The Berners Bay population is stable and thought to be close to carrying capacity at 120-150 individuals (Barten 2008). Both populations support limited harvest.

The moose population in Yukon Flats (66º 10' N, 149º 00' W) occurs in lowland boreal forest along the Yukon River in central Alaska. Although the population exists in a large contiguous area of suitable moose habitat, it occurs at extremely low density (Caikoski 2008) presumably due to predation (Bertram and Vivion 2002) and poaching of female moose (Caikoski 2008). Nonetheless, Yukon Flats is an open population as opposed to the presumably closed nature of the Berners Bay and Kalgin Island populations. Although the Yukon Flats population was not the source of founders for either Berners Bay or Kalgin Island, it serves as a good example of an outbred Alaskan moose population and should serve as a suitable control population in lieu of samples from south-central Alaska. Indeed, Schmidt et al. (2009) found little difference in levels of diversity among 6 moose populations distributed widely within Alaska, demonstrating that location of the population is less important than demographic history.

METHODS

Samples for genetic analysis were acquired either as tissue from hunters (Kalgin Island and Berners Bay) or as blood samples from captured animals (Yukon Flats). Samples for each population were collected within a single year. DNA extraction and genotyping were conducted under contract in one of two laboratories: Kalgin Island and Berners Bay samples were analyzed at Wildlife Genetics International (Nelson, British Columbia, Canada), whereas Yukon Flats moose were analyzed at the Department of Biological Sciences, University of Alberta (Calgary, Alberta, Canada). Two samples from Yukon Flats were also analyzed by Wildlife Genetics International to ensure consistency in allele calling and warrant comparison of genotypes from the two labs. I analyzed 19 samples from Kalgin Island, 8 from Berners Bay, and 28 from Yukon Flats.

Loci BL42, BM4513, BM888, BM1222, BM203, BM848 (Bishop et al. 1994), FCB193 (Buchanan and Crawford 1993), Rt5, Rt9, Rt24, and Rt30 (Wilson et al. 1997) were used to characterize genetic diversity. Populations were tested to ensure compliance with Hardy-Weinberg equilibrium using an exact chi-

square test implemented in software GENEPOP (Raymond and Rousset 1995). Diversity was expressed as allelic richness (number of alleles per locus, A), observed heterozygosity (H_O) and expected heterozygosity (H_E) based on allele frequencies assuming Hardy-Weinberg equilibrium. Because estimates of allelic richness are related to sample size, we standardized our estimates by using rarefaction (Kalinowski 2005) to express the expected number of alleles per locus based on a sample of 8 individuals from each population (the smallest sample size of our 3 populations). Estimates of allelic richness were compared between population pairs using a sign test. Estimates of inbreeding (F_{IS}) were calculated by GENEPOP.

Population differentiation was assessed via Nei's unbiased genetic distance (Nei 1978), pairwise F_{ST}, and comparison of allele frequencies of populations. Significance of differences based on pairwise F_{ST} estimates was estimated from a permutation test conducted in software FSTAT (Goudet 1995). Significance of pairwise comparisons of allele frequencies was conducted as a chi-square test for each locus and significance values were combined via Fisher's method (Fisher 1948) by GENEPOP to compute a population-wide significance level.

RESULTS AND DISCUSSION

All populations were in Hardy-Weinberg equilibrium (Kalgin Island: $\chi^2 = 15.4$, $P = 0.75$; Berners Bay: $\chi^2 = 24.5$, $P = 0.14$; Yukon Flats: $\chi^2 = 18.8$, $P = 0.66$). All loci were polymorphic in Yukon Flats whereas one locus (Rt9) was monomorphic in both Kalgin Island and Berners Bay populations. All populations differed from each other in allelic richness ($P < 0.001$; Table 1). The 3 populations shared at least one allele at each locus, but allele frequencies differed in pairwise comparisons ($P < 0.0001$). The extent of the reduction in diversity undergone by Kalgin Island and Berners Bay populations is illustrated by the occurrence of private alleles (those occurring in only one population; Table 1), wherein Yukon Flats had 17 alleles not found in the other populations. Kalgin Island exhibited the least diversity, as measured by allelic richness and heterozygosity, followed by Berners Bay and Yukon Flats (Table 1). Inbreeding coefficients for all populations were very close to zero, indicating no evidence of inbreeding. All populations differed from each other in pairwise comparisons of genetic distance and F_{ST} (Table 2); the largest differences were between the 2 introduced populations. It is striking that Kalgin Island and Berners Bay populations differed to such a degree considering that their founding individuals came from the same general area.

Sample size of the Berners Bay population was less than ideal but the samples were obtained opportunistically and there was a low probability of obtaining additional samples from the few hunters that harvest moose there. Nonetheless, estimates of observed and expected heterozygosity, as well as F_{IS} and F_{ST} that are based on heterozygosity, are not related to sample size and would not be expected to change predictably with an increase

Table 1. Indices of genetic diversity for 11 microsatellite loci measured in 3 Alaskan moose populations. Kalgin Island and Berners Bay populations show limited diversity due to founder events and lack of gene flow with neighboring populations. Parameters are: n = sample size, A = allelic richness (alleles/locus), A_8 = estimate of A standardized to a sample size of 8, H_O = observed heterozygosity, H_E = expected heterozygosity, and F_{IS} = inbreeding coefficient.

Population	n	A	A_8	Private alleles	H_O	H_E	F_{IS}
Kalgin Island	19	2.9	2.7	1	0.47	0.45	-0.01
Berners Bay	8	3.1	3.1	2	0.53	0.49	-0.03
Yukon Flats	28	5.5	4.2	17	0.67	0.64	-0.02

in sample size. Of the indices of diversity that I examined, only allelic richness is affected by sample size (Kalinowski 2005, Pruett and Winker 2008), which is why I used a rarefaction method so that estimates of richness among populations could be compared. Results from a simulation study indicated that mean estimates of H_O and H_E did not change significantly for sample sizes ranging from 5-100 individuals, and that estimates were more consistent over a range of sample sizes for populations with low genetic diversity as compared with high diversity (Pruett and Winker 2008).

Clearly, moose populations in Kalgin Island and Berners Bay show severely reduced diversity relative to Yukon Flats, a result that arguably stems from genetic bottlenecks associated with introduction. Moreover, Kalgin Island moose were significantly less diverse that Berners Bay moose; this difference is likely a function of the smaller founding size of the Kalgin Island population combined with its marked fluctuations.

Genetic differentiation among the 3 populations was much greater than that reported among 6 moose populations from Alaska (F_{ST} range = 0.014-0.109; Schmidt et al. 2009). That study found a remarkable lack of differentiation among moose across a large geographic scale, and was contrary to other studies (Broders et al. 1999, Wilson et al. 2003). The relative difference among the 3 study populations would be unexpected if the Kalgin Island and Berners Bay populations were open and exchanged individuals with neighboring populations. Thus, these differences presumably indicate the strong effect of founding combined with genetic drift associated with isolation from neighboring populations.

Reduction in heterozygosity associated with founder effect can be calculated as:

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

Where: H_O is the heterozygosity of the source population, H_E is the expected heterozygosity of the founded population at the time of founding, and N is the number of individuals introduced. Using Yukon Flats as a proxy for the source population (H_O = 0.67; Table 1), Kalgin Island with a founding size of 6 would have H_E = 0.61 which is 30% greater than H_O for Kalgin (0.47; Table 1). Similarly, H_E for the initial population in Berners Bay would be expected to be 0.66 or 24% higher than observed (0.53; Table 1). The differences between the current heterozygosity in the introduced populations and the expected heterozygosity based on number of founders can be explained by genetic drift occurring in the interim. Moreover, the severity of a genetic bottleneck is directly related to the duration of the bottleneck (Nei et al. 1975), suggesting that both populations grew slowly after founding, thus extending the length of the bottleneck; the Kalgin Island population was likely affected by a second bottleneck when the population declined abruptly to the founding size (8 individuals) in the 1980s.

Table 2. Indices of population differentiation based on 11 microsatellite markers measured in 3 Alaskan moose populations. Nei's (1978) unbiased genetic distance is above the diagonal and F_{ST} is below the diagonal. Based on F_{ST} estimates, all populations differ significantly ($P < 0.001$).

	Kalgin Island	Berners Bay	Yukon Flats
Kalgin Island		0.518	0.365
Berners Bay	0.301		0.31
Yukon Flats	0.197	0.151	

Interpopulation distances reported for moose in Canada (Broders et al. 1999) were much lower than those reported here; distances were 0.013-0.298, however the latter value was essentially a comparison of 2 different subspecies representing moose from Cape Breton Island, Nova Scotia (introduced from Alberta) and moose from the Avalon Peninsula, Newfoundland (introduced from Nova Scotia

and New Brunswick). Distances reported by Wilson et al. (2003) were comparable to those reported here; however, the largest F_{ST} value (0.3013) occurred between populations in Newfoundland and Riding Mountain National Park, Manitoba and was a comparison across a subspecies boundary. Thus, the level of differentiation observed between the 2 introduced Alaska populations was equal to or greater than that observed between subspecies elsewhere on the continent. Comparisons of levels of diversity between studies employing different sets of molecular markers require caution because of the different levels of variation inherent in different loci. Nonetheless, if loci are truly neutral and markers conform to the same model of mutation, estimates of population differentiation should be broadly comparable between studies.

I have shown that 2 small, introduced moose populations in Alaska underwent extreme reductions in genetic diversity associated with founding and subsequent genetic drift. It is highly unlikely that either population experiences gene flow with neighboring populations; otherwise, the level of diversity in the introduced populations would be greater and more similar with that of Yukon Flats. Managers of the Kalgin Island and Berners Bay populations should consider the degree of isolation and paucity of genetic variation in those populations when assessing risks to population viability and crafting management strategies. As an example, recovery of diversity can probably be accomplished only with introduction of additional individuals rather than relying on immigration from surrounding populations.

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