# POPULATION GENETIC STRUCTURE OF MOOSE (*ALCES ALCES*) OF SOUTH-CENTRAL ALASKA

**Robert E. Wilson[1,2], Thomas J. McDonough[3], Perry S. Barboza[1], Sandra L. Talbot[4], and Sean D. Farley[5]**

[1]University of Alaska Fairbanks, Institute of Arctic Biology, Fairbanks, Alaska 99775; [3]Alaska Department of Fish and Game, 3298 Douglas St., Homer, Alaska 99603; [4]U. S. Geological Survey, Alaska Science Center, 4210 University Drive, Anchorage, Alaska 99508; [5]Alaska Department of Fish and Game, 333 Raspberry Road, Anchorage, Alaska 99518

ABSTRACT: The location of a population can influence its genetic structure and diversity by impacting the degree of isolation and connectivity to other populations. Populations at range margins are often thought to have less genetic variation and increased genetic structure, and a reduction in genetic diversity can have negative impacts on the health of a population. We explored the genetic diversity and connectivity between 3 peripheral populations of moose (*Alces alces*) with differing potential for connectivity to other areas within interior Alaska. Populations on the Kenai Peninsula and from the Anchorage region were found to be significantly differentiated ($F_{ST}$ = 0.071, $P < 0.0001$) with lower levels of genetic diversity observed within the Kenai population. Bayesian analyses employing assignment methodologies uncovered little evidence of contemporary gene flow between Anchorage and Kenai, suggesting regional isolation. Although gene flow outside the peninsula is restricted, high levels of gene flow were detected within the Kenai that is explained by male-biased dispersal. Furthermore, gene flow estimates differed across time scales on the Kenai Peninsula which may have been influenced by demographic fluctuations correlated, at least in part, with habitat change.

ALCES VOL. 51: 71–86 (2015)

**Key words**: Alaska, genetic diversity, gene flow, moose, population genetic structure

The pattern of geographical variation in genetic diversity and divergence is dictated by the interaction of genetic drift, gene flow, and natural selection (Eckert et al. 2008), and these evolutionary processes can be influenced by the location of a population within the species' geographic range (Briggs 1996, Wisely et al. 2004, Howes and Lougheed 2008). At the local and regional scales, the relative location of a population can strongly impact patterns of dispersal and degree of isolation influenced by both historical and contemporary events (Vucetich and Waite 2003, Eckert et al. 2008), ultimately determining the level of genetic structure and diversity. Genetic diversity is lowest at the range margins and highest at the center of a species distribution (Yamashita and Polis 1995, Schwartz et al. 2003, Eckert et al. 2008, Howes and Lougheed 2008). Marginal populations are more likely to be isolated, occur in patchy habitats, and may reflect recent colonization. Peripheral populations are less likely to receive immigrants whereas the core populations typically occupy prime habitat and experience greater levels of gene flow (Hoffmann and Blows 1994, Brown et al. 1995, Wisely et al. 2004, Miller et al. 2010, Schrey et al. 2011).

Evolutionary theory suggests that the reduction of genetic diversity within peripheral populations can impede adaptation to

[2]Present address: U. S. Geological Survey, Alaska Science Center, 4210 University Drive, Anchorage, Alaska 99508

changing environmental conditions (Bradshaw 1991, Hoffmann and Parsons 1991, Hoffmann and Blows 1994, Blows and Hoffmann 2005). Such adaptation is largely determined by the availability of additive genetic variation in heritable traits with fitness consequences. Several studies have shown that even small changes in genetic variation can have large effects on population fitness (Frankham 1995, Reed and Frankham 2003) including juvenile survival (Coulson et al. 1999, Mainguy et al. 2009, Silva et al. 2009), antler growth (Von Hardenberg et al. 2007), and parasite resistance (Coltman et al. 1999) within ungulates. However, adaptability is also affected by effective dispersal which can have either positive or negative effects on the population depending on the rate of gene flow and the strength of selection acting on the local population (García-Romos and Kirkpatrick 1997, Akerman and Bürger 2014, Bourne et al. 2014, Frankham 2015). Thus, examining conditions (habitat, genetic diversity, gene flow rates, and life history) under which peripheral populations exist can aid in understanding the processes that maintain geographical ranges, predicting the consequences of climate change (Parmesan and Yohe 2003, Root et al. 2003, Hampe and Petit 2005), and conserving populations at range margins (Howes and Lougheed 2008).

The Kenai Peninsula is a peripheral region situated in south-central Alaska that was separated from the mainland by a narrow (16 km wide) isthmus at the end of the last ice age. Due to its diverse landscape, biodiversity in this region is unusually high at this latitude (Morton et al. 2009), and the moose (*Alces alces*) is one of the most recognizable and socio-economically important species. Moose populations on the Kenai Peninsula are characterized by fluctuations in population size, peaking after the occurrence of forest fires that promote optimal forage habitat (Oldemeyer et al. 1977). While moose populations on the Kenai have fluctuated between 5,000–8,000 animals over the past several decades (T. J. McDonough, unpublished data), these fluctuations have not been uniform across moose management units on the peninsula. While population size in Game Management Unit (GMU) 15C in southwest Kenai has increased, numbers in GMU 15A (northwest) have declined drastically, ~40% in the last 20 years as quality forage has diminished since the last major fire in 1969. Relative isolation from neighboring regions with a strong history of fluctuations in population size might lead to reduced genetic variability on the Kenai Peninsula which could ultimately be detrimental to the long-term health of moose in this region.

Using microsatellite loci, we compared levels of genetic variation and gene flow in 2 areas within the Kenai Peninsula and the Anchorage area. These 3 areas are situated on the periphery of overall moose distribution in Alaska but differ in levels of potential connectivity to the core area of interior Alaska. First, we investigated the connectivity between GMUs on the Kenai Peninsula that have been affected by a long history of land alteration and demographic changes. Second, we predicted that 2 sites within the disjunct Kenai Peninsula region, where opportunities for genetic exchange may be more limiting than Anchorage, would exhibit relatively lower genetic diversity.

## METHODS

### Sample collection

A total of 163 moose were sampled from 3 populations in south-central Alaska (Fig. 1). Ear-plugs and blood were taken from 33 collared female moose in 2008–2010 and 2012 from the city of Anchorage and adjacent Eagle River (called Anchorage hereafter). In addition, muscle tissue was taken from 32 hunter-killed moose (16 female, 15 male, and 1 unknown) during the winter of

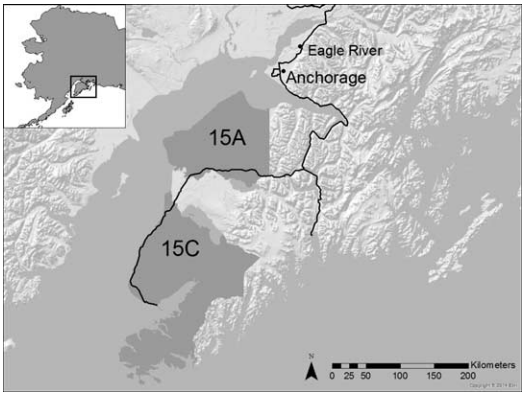


Fig. 1. Sampling areas for three moose populations in south-central Alaska: Anchorage, Game Management Unit (GMU) 15A (northwest Kenai Peninsula), and GMU15C (southwest Kenai Peninsula).

2011–2012. In spring 2012, blood was taken from radio-collared female moose from GMU 15A (n = 49; 3,367 km$^2$) and GMU 15C (n = 49; 3,030 km$^2$) on the Kenai Peninsula, the borders of which are approximately 20 km apart. Anchorage samples are archived at the Molecular Ecology Laboratory, U.S. Geological Survey, Anchorage, Alaska, and Kenai Peninsula samples at the Alaska Department of Fish and Game, Homer, Alaska. All animal capturing and genetic sampling were conducted under Division of Wildlife Conservation ACUC approval (# 2012–2007, 2013–2021, and 90–05) and under the University of Alaska Fairbanks IACUC approval (# 14885 and 182744).

## Molecular techniques

Genomic DNA was extracted from blood and tissue samples using a "salting out" procedure described by Medrano et al. (1990), with modifications described in Sonsthagen et al. (2004). Genomic DNA concentrations were quantified using fluorometry and diluted to 50 ng mL$^{-1}$ working solutions. Individuals were initially screened at 17 microsatellite loci. Thirteen autosomal loci were found to be polymorphic of which 9 with dinucleotide repeat motifs were selected for further analysis that were polymorphic in all populations: BL42, BM888, BM203, BM2830 (Bishop et al. 1994), NVHRT21, NVHRT22 (Røed and Midthjell 1998), RT1, RT5, and RT30 (Wilson et al. 1997). Polymerase chain reaction (PCR) amplification and electrophoresis followed protocols described in Roffler et al. (2012). Ten percent of the samples were amplified and genotyped in duplicate for the 9 microsatellite loci for quality control.

## Analysis of genetic diversity and population genetic subdivision

We calculated allelic richness, inbreeding coefficient ($F_{IS}$), observed and expected heterozygosities, and tested for deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) for each microsatellite locus and population in FSTAT ver. 2.9.3 (Goudet 1995). The degree of genetic subdivision among moose populations was assessed by calculating overall and pairwise $F_{ST}$ and $R_{ST}$, adjusting for multiple comparisons using Bonferroni correction ($\alpha = 0.05$) in Arlequin v3.5.1.3 (Excoffier and Lischer 2010). Because the upper possible $F_{ST}$ value for a set of microsatellite loci is usually <1.0 (Hedrick 2005), we used RECODEDATA, version 1.0 (Meirmans 2006) to calculate the uppermost limit of $F_{ST}$ for our data set.

We also used the Bayesian-clustering program STUCTURE 2.2.3 (Pritchard et al. 2000) to determine the level of population structure in the autosomal microsatellite data set. We performed 2 sets of analyses to look at structure within south central Alaska: 1) between Anchorage and Kenai Peninsula and 2) within the Kenai Peninsula (GMU 15A and 15C). Structure assigns individuals to populations maximizing Hardy-Weinberg equilibrium and minimizing linkage disequilibrium. The analysis was conducted for 1–10 populations (K) using an admixture

model with 100,000 burn-in iterations and 1,000,000 Markov chain Monte Carlo (MCMC) iterations without providing *a priori* information on the geographic origin of the individuals; the analyses were repeated 10 times for each K to ensure consistency across runs. We used the ΔK method of Evanno et al. (2005) and evaluation of the estimate of the posterior probability of the data given K, Ln P(D), to determine the most likely number of groups at the uppermost level of population structure. For the Kenai Peninsula analysis we used the LOCPRIOR which is able to detect weak signals of population structure in datasets not detectable under standard models (Hubisz et al. 2009). We determined if location was informative by the value of *r*, which parameterizes the amount of information contained by the location of the samples. Values of $r > 1$ indicates either there is no population structure or that structure is independent of locality.

**Gene flow**

We estimated gene flow between moose populations using 2 methodologies: MIGRATE v3.2.16 (Beerli and Felsenstein 1999, 2001) and BayesAss 3.0 (Wilson and Rannala 2003). These programs differ in the underlying model used to estimate gene flow. MIGRATE uses a steady-state two-island coalescent model of population differentiation which incorporates parameters scaled to the mutation rate (μ), the effective population size parameter Θ ($4N_e\mu$), and the migration rate M (m/μ) between populations. BayesAss uses an assignment methodology which does not incorporate genealogy or assume that populations are in Hardy-Weinberg equilibrium (Wilson and Rannala 2003). Thus, estimates of migration rate can be interpreted differently and at different temporal scales. BayesAss reflects gene flow over the last several generations (referred to as contemporary gene flow hereafter) whereas MIGRATE gene flow estimates are averaged over the past *n* generations, where *n* equals the number of generations the populations have been at mutation-drift equilibrium (Beerli and Felsenstein 1999, 2001). It is generally agreed that microsatellite mutation rates are several orders of magnitude higher than mutation rates of DNA sequences (mitochondrial or nuclear; Schlötterer 2000, Ellegren 2004). Thus, microsatellite markers can reflect recent (within the last 10,000 years) and almost contemporaneous events, but increases in homoplasy associated with microsatellites reduce their ability to capture older demographic events (Hartl and Clark 2007, Hughes 2010). Therefore, MIGRATE analyses are referred to as estimating recent gene flow.

MIGRATE was run with a full migration model; θ ($4N_e\mu$, composite measure of effective population size and mutation rate) and all pairwise migration parameters were estimated individually from the data. Gene flow was estimated using maximum likelihood search parameters; 10 short chains (5000 trees used out of 1,500,000 sampled), 10 long chains (15,000 trees used out 5,250,000 sampled), and 5 static heated chains (1, 1.33, 2.0, 4.0, and 1,000,000; swapping interval = 1). Full models were run 10 times to ensure the convergence of parameter estimates.

BayesAss was initially run with the default delta values for allelic frequency (P), migration rate (m), and inbreeding (F). Subsequent runs incorporated different delta values to ensure that acceptance rate for proposed changes was between 20–40% for each parameter to maximize log likelihood values and ensure the most accurate estimates (Wilson and Rannala 2003). Final delta values used were ΔP = 0.5 (27% acceptance rate), Δm = 0.2 (27%), and ΔF = 0.85 (31%). We performed 10 independent runs (10 million iterations, 1 million burn-in,

and sampling frequency of 1000) and 2 additional longer runs (50 million iterations, 5 million burn-in) with different random seeds to ensure convergence and consistency across runs. Convergence was also assessed by examining the trace file in program Tracer v1.5 to ensure proper mixing of parameters (Rambaut and Drummond 2007).

### Population demography

To estimate the effective population size (*Ne*) for each GMU on the Kenai Peninsula and Anchorage area, we used the approximate Bayesian computation method (Beaumont et al. 2002) implemented in the program ONeSAMP 1.2 (Tallmon et al. 2008). We used a lower prior of 100 for all populations and a maximum prior that reflected the current census size (1,000 for Anchorage, 2,000 for GMU 15A, and 3,000 for GMU 15C). Similar values were obtained for larger maximum possible effective population sizes.

Lastly, we used BOTTLENECK which compares the number of alleles and gene diversity at polymorphic loci under the infinite allele model (IAM; Maruyama and Fuerst 1985), stepwise mutation model (SMM; Ohta and Kimura 1973), and two-phase model of mutation (TPM; Di Rienzo et al. 1994; parameters: 79% SSM, variance 9; Piry et al. 1999, Garza and Williamson 2001). One thousand simulations were performed for each population and parameters were changed among 5 runs to evaluate the robustness of results. Significance was assessed using a Wilcoxon sign-rank test which determines if the average of standardized differences between observed and expected heterozygosities is significantly different from zero (Cornuet and Luikart 1996). Significant heterozygote deficiency relative to the number of alleles indicates recent population growth, whereas heterozygote excess relative to the number of alleles indicates a recent population bottleneck (Cornuet and Luikart 1996). BOTTLENECK compares heterozygote deficiency and excess relative to genetic diversity, not to Hardy-Weinberg equilibrium expectation (Cornuet and Luikart 1996).

## RESULTS

### Genetic diversity and population subdivision

Multilocus genotypes were collected from 163 individuals and each individual had a unique genotype. The number of alleles per locus observed ranged from 3.4–4.7 per population with an overall estimate of 5.1 (Tables 1, 2). The observed heterozygosity ranged from 43–55% with an overall mean heterozygosity of 49%. The Kenai Peninsula exhibited a 19% lower allelic richness (20% in GMU 15A and 25% in GMU 15C) compared to the Anchorage area, and 3x more private alleles were observed in the Anchorage region (Table 1). In addition, the observed ($H_o$) and expected ($H_e$) heterozygosity was significantly lower (all *P*-values < 0.0001) in the Kenai Peninsula ($H_o$ by 18%, $H_e$ by 16% expected), in GMU 15C (15%, 14%), and in GMU 15A (22%, 20%). On average, individuals on the Kenai showed a greater level of homozygosity (Kenai: 4.94 loci [SD = 1.46] vs. Anchorage: 3.98 loci [SD = 1.51]; $t = 4.02$, $P < 0.0001$). The inbreeding coefficient ($F_{IS}$) did not differ significantly from zero in any population (Table 1). All loci and populations were in HWE and linkage equilibrium.

Significant genetic structure was observed at the 9 microsatellite loci between Anchorage and the two GMUs on the Kenai Peninsula (Table 3). No significant difference was found within the Kenai Peninsula. The upper limit of the $F_{ST}$ for our microsatellite data set was 0.499. Therefore, the overall $F_{ST}$ of 0.071 accounted for 14.2% of the maximum possible level of genetic structure and 19% for the pairwise

Table 1. Estimates of genetic diversity of the moose sampled from three locales in south-central Alaska, including: average number of alleles, allelic richness ($A_R$), observed and expected heterozygosities ($H_o/H_e$), inbreeding coefficient ($F_{IS}$), effective population size ($N_e$) estimated in ONeSAMP and sample size ($n$) calculated from nine microsatellite loci. Allelic richness is based on smallest sample size of 65 for Anchorage and overall Kenai. Within Kenai Peninsula (GMU 15A and GMU 15C) based on sample size of 49.

| | | Kenai Peninsula | | |
|---|---|---|---|---|
| | Anchorage | GMU 15A | GMU 15C | Overall Kenai |
| No. Alleles | 4.67 | 3.67 | 3.44 | 4.00 |
| No. Private Alleles | 10 | 1 | 2 | 3 |
| $A_R$ | 4.59 | 3.67 | 3.44 | 3.78 |
| $H_o$ (SD) / $H_e$ (SD) | 0.55 (0.02)/ 0.56 (0.06) | 0.43 (0.02)/ 0.45 (0.07) | 0.47 (0.02)/ 0.48 (0.05) | 0.45 (0.02)/ 0.47 (0.06) |
| $F_{IS}$ | 0.007 | 0.056 | 0.031 | 0.043 |
| $N_e$ | 74.3 (67.6–83.0) | 47.9 (43.2–56.8) | 36.8 (33.3–43.9) | 145.5 (123.3–255.9) |
| n | 65 | 49 | 49 | 98 |

comparisons between Anchorage and Kenai Peninsula GMUs.

STRUCTURE uncovered genetic partitioning within south central moose populations, supporting a two-population model ($\Delta K = 188.3$, average Ln P(D) = −2758.7). Most individuals from Anchorage were assigned to one genetic cluster (87.7%), whereas individuals from Kenai GMU 15A and 15C were assigned to a second cluster with high probability, 93.6 and 92.6%, respectively (Fig. 2). Seven Anchorage individuals were assigned to the Anchorage cluster with <60% certainty, conversely, only a single Kenai individual was assigned to the Kenai cluster with <60% certainty. Genetic partitioning was not observed within Kenai Peninsula, as including capture location (LOCIPRIOR) was not informative ($r > 9$).

## Gene flow

Restricted gene flow over the past several generations was observed under the BayesAss model between Anchorage and Kenai Peninsula, with 96.8% (93.3–100%) of the Anchorage population comprised of a non-migrant origin (Fig. 3). Within the Kenai Peninsula, there was a signal of a northern direction of contemporary gene flow from GMU 15C into 15A (proportion of individuals with migrant origin: 27.8% in 15A vs. 6.9% in 15C); although the 95% confidence intervals do overlap (Fig. 3).

Asymmetrical recent gene flow as estimated by MIGRATE was observed among sampled populations. The directionality of gene flow was from Kenai Peninsula into Anchorage (Fig. 3). The number of migrants per generation ($N_em$) ranged from 2.56 (GMU 15A; 1.97–3.29) and 2.78 (GMU 15C; 2.20–3.48) into Anchorage and 0.99 (0.74–1.32) and 1.08 (0.78–1.47) into the Kenai GMU 15A and 15C, respectively. Within Kenai there was a signal of asymmetrical gene flow from GMU 15A into 15C (3.3 migrants/generation; Fig. 3).

## Population demography

The estimated effective size using the Bayesian computation method for the Anchorage region was 74.3 (95% CI: 67.6–83.0). GMUs 15A and 15C on the Kenai Peninsula had lower estimated effective sizes with non-overlapping confidence intervals with Anchorage (Table 1). The BOTTLENECK analysis showed no evidence of significant

Table 2. Estimates of observed and expected heterozygosity, number of alleles per locus for nine autosomal nuclear microsatellite loci assayed in three moose populations in south-central Alaska. All loci were in Hardy-Weinberg equilibrium. Sample size is in parentheses; $H_o$ = heterozygosity observed, $H_e$ = heterozygosity expected, and $N_a$ = number of alleles.

| | | | Kenai Peninsula | | | |
|---|---|---|---|---|---|---|
| Locus | | Anchorage (65) | GMU 15A (49) | GMU 15C (49) | Overall Kenai (98) | All populations (165) |
| NVHRT22 | $H_o/H_e$ | 0.69/0.76 | 0.49/0.54 | 0.57/0.53 | 0.53/0.53 | 0.60/0.68 |
| | $N_a$ | 6 | 5 | 4 | 6 | 6 |
| NVHRT21 | $H_o/H_e$ | 0.49/0.50 | 0.55/0.46 | 0.39/0.45 | 0.47/0.45 | 0.48/0.48 |
| | $N_a$ | 5 | 3 | 2 | 3 | 5 |
| RT1 | $H_o/H_e$ | 0.49/0.46 | 0.27/0.29 | 0.39/0.38 | 0.31/0.34 | 0.38/0.40 |
| | $N_a$ | 2 | 2 | 2 | 2 | 2 |
| RT5 | $H_o/H_e$ | 0.54/0.52 | 0.18/0.21 | 0.25/0.32 | 0.21/0.26 | 0.34/0.40 |
| | $N_a$ | 4 | 3 | 3 | 3 | 4 |
| RT30 | $H_o/H_e$ | 0.55/0.58 | 0.69/0.67 | 0.74/0.72 | 0.71/0.70 | 0.65/0.66 |
| | $N_a$ | 5 | 4 | 4 | 4 | 5 |
| BM203 | $H_o/H_e$ | 0.20/0.20 | 0.37/0.41 | 0.51/0.50 | 0.44/0.46 | 0.34/0.38 |
| | $N_a$ | 5 | 3 | 4 | 4 | 6 |
| BM2830 | $H_o/H_e$ | 0.46/0.49 | 0.37/0.43 | 0.41/0.41 | 0.39/0.42 | 0.42/0.45 |
| | $N_a$ | 2 | 2 | 2 | 2 | 2 |
| BM888 | $H_o/H_e$ | 0.63/0.65 | 0.22/0.26 | 0.20/0.27 | 0.21/0.27 | 0.38/0.46 |
| | $N_a$ | 4 | 4 | 3 | 4 | 4 |
| BL42 | $H_o/H_e$ | 0.91/0.84 | 0.71/0.81 | 0.80/0.75 | 0.76/0.79 | 0.82/0.83 |
| | $N_a$ | 9 | 6 | 7 | 8 | 12 |
| Overall Loci | $H_o/H_e$ | 0.55/0.56 | 0.43/0.45 | 0.47/0.48 | 0.45/0.47 | 0.49/0.53 |
| | $N_a$ | 4.67 | 3.67 | 3.44 | 4.00 | 5.11 |

Table 3. Pairwise and overall values of $F_{ST}$ and $R_{ST}$ calculated from nine microsatellite loci. Significant values after Bonferroni correction ($P < 0.0001$) are marked with an asterisk.

| | $F_{ST}$ | $R_{ST}$ |
|---|---|---|
| Anchorage | | |
| – Kenai GMU 15A | 0.094* | 0.014 |
| – Kenai GMU 15C | 0.092* | 0.028 |
| Kenai GMU 15A | | |
| – Kenai GMU 15C | 0.001 | 0.000 |
| Overall | 0.071* | 0.016 |

heterozygosity excess or deficit under the SMM or TPM. However, there was evidence of a recent population decline (heterozygote excess) based on the infinite allele model (IAM) for Kenai GMU 15C.

## DISCUSSION

Climatic and glaciation history has played a major role in shaping the evolutionary history of many taxa in south-central Alaska. It was not until approximately 7,000 years before present that the Kenai Peninsula became distinct and relatively isolated from the mainland by a 16 km wide mountainous isthmus (Pielou 1991, Muhs et al. 2001). This isolation has fostered genetically or morphologically distinct populations for a variety of taxa (e.g., wolverine

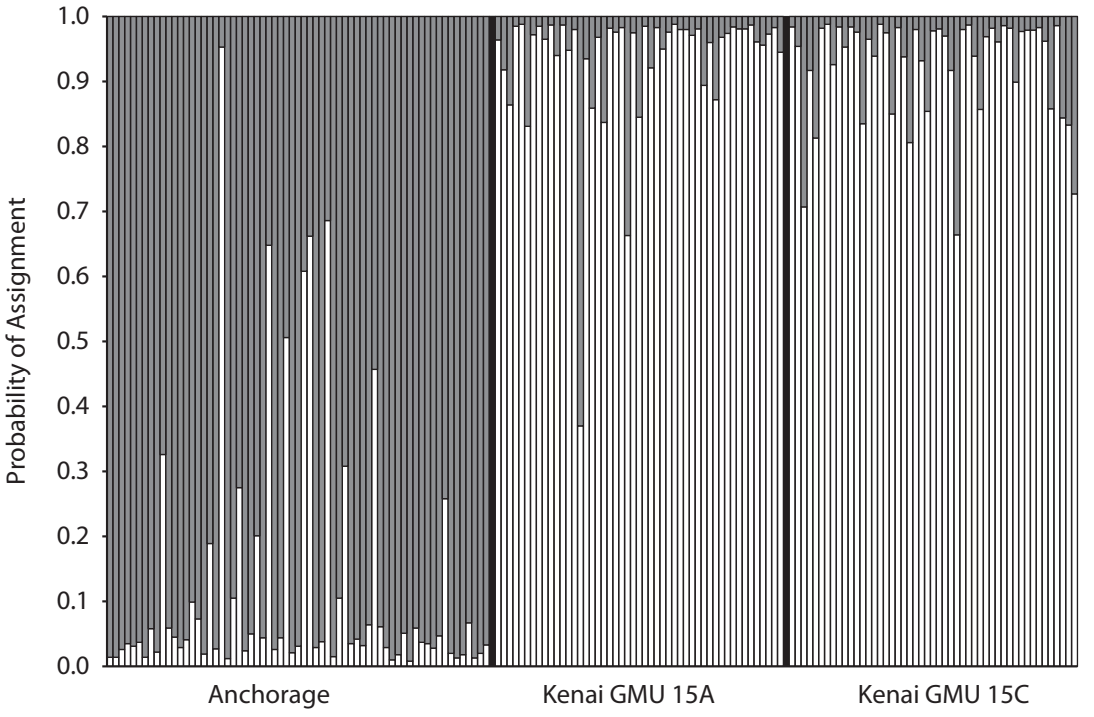


Fig. 2. Structure analysis showing posterior probability of assignment of individuals to each ($K$ = 2) genetic cluster. White bar represents the estimated probability of assignment to cluster one and grey bar is the estimated probability of assignment to cluster two.

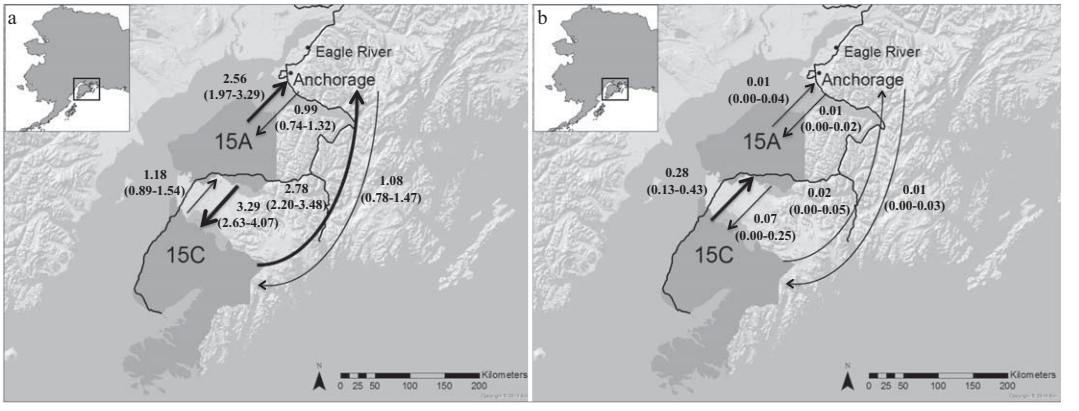


Fig. 3. Estimates of (a) recent (number of migrants per generation, $N_em$) estimated in MIGRATE and (b) contemporary (proportion of individuals with migrant origin, $m$) calculated in BayesAss for moose populations in south-central Alaska as calculated from nine microsatellite loci with relative magnitude indicated by width of arrow; 95% confidence intervals are in parentheses.

[*Gulo gulo*], Tomasik and Cook 2005; American marten [*Ursus americanus*], Robinson et al. 2007; song sparrow [*Melospiza melodia*], Patten and Pruett 2009). The moose populations residing on the Kenai are no exception. Using a multi-locus approach, we observed that moose on the Kenai were genetically distinct from those in the

mainland Anchorage population and exhibited significantly lower levels of genetic diversity at microsatellite loci.

### Loss of genetic diversity between peninsula and mainland

Populations residing in areas with barriers that limit dispersal (e.g., peninsulas and islands) across the landscape are expected to have lower genetic variation (Gaines et al. 1997). Our results were consistent with Gaines et al. (1997) prediction: moose occupying the Kenai Peninsula had significantly reduced genetic diversity (~18%) compared to the nearest mainland population in Anchorage. A reduction of genetic variability has also been reported for other Alaskan moose populations (Hundertmark 2009, Schmidt et al. 2009) as well as other mammals on the Kenai Peninsula (e.g., Canada lynx [*Lynx canadensis*], Schwartz et al. 2003). The loss of genetic variation in peripheral populations may be due to numerous factors such as limited number of connections to other populations or smaller population size (Schwartz et al. 2003).

Cook Inlet waters, mountains, and a highway and railways may represent formidable dispersal barriers for moose between these regions. Although Kenai Peninsula and Anchorage are in close geographic proximity (straight line distance over land is ~105 km), the costs of dispersal over the rugged terrain and highways or swimming across the inlet are likely high. In agreement with limited effective dispersal, we found restricted contemporary gene flow between Kenai Peninsula and mainland Anchorage populations with confidence intervals suggesting there has been limited genetic exchange over the past several generations. Telemetry studies of the sampled females in this study showed that individuals remained in the same general area throughout the year (Farley et al. 2012, T. J. McDonough, unpublished data), further suggesting a low likelihood of long-distance dispersal between these two regions.

However, connectivity could be mediated through a contact zone north of the isthmus located at Portage Valley that is used by black bears (*Ursus americanus*) (Robinson et al. 2007). The isthmus is not an absolute/strong barrier as movements of radio-collared moose occur across the isthmus; this movement was restricted within intermountain valleys that spanned both sides of the isthmus (T. Lohuis, Alaska Department of Fish and Game, unpublished data). Furthermore, STRUCTURE analysis estimated a low probability assignment to a genetic cluster for ~12% of the individuals in Anchorage, suggestive of genetic exchange that has occurred during or after population divergence, with higher rate going into the Anchorage area based on the MIGRATE analysis. This northward direction of gene flow is also found in other peninsular populations (Schmidt et al. 2009) and may reflect post-colonization gene flow rates. Further study of moose in areas between Anchorage and Kenai Peninsula might identify if a contact zone exists for moose at the isthmus as seen in other mammals, or if these regions are truly isolated as indicated by the contemporary gene flow analysis.

### Relationships within the peninsula

Unlike the potential strong barriers to dispersal between the peninsula and mainland populations, there are relatively few natural barriers to movement in the western part of the peninsula, and gene flow estimates suggest that there is ongoing genetic exchange. The directionality of gene flow across the western Kenai Peninsula has not remained constant over time. Differences in directionality across time scales may be attributed to the fluctuating nature of moose population dynamics that is correlated at least in part with habitat change, in particular

in GMU 15A where population size fluctuates with major fire events (Oldemeyer et al. 1977, Schwartz and Franzmann 1989, Loranger et al. 1991). The habitat in GMU 15A has changed drastically over the last century after major fires in 1947 and 1969 transformed previously low quality habitat to ideal foraging habitat, which subsequently declined to the current condition (Oldemeyer et al. 1977, Schwartz and Franzmann 1989). If periodic population increase has been sufficiently frequent throughout the history of moose in this area, and dispersal is influenced by population density and habitat quality, we might expect the directionality of gene flow to change over time with more moose dispersing from areas of high productivity into areas of lower density or less preferred habitat as competition for resources increases. Indeed, contemporary gene flow estimated in BayesAss indicates gene flow from a higher density area (GMU 15C) with better quality habitat into an area characterized by poor habitat conditions and lower density area (15A).

Moose populations on the Kenai Peninsula have also fluctuated in size partially due to human activities (land development and forest fires), with changes in habitat potentially affecting fertility and survival of young (Klein 1970, Franzmann and Arneson 1973, Schwartz and Franzmann 1989, Testa and Adams 1989). While moose populations initially respond positively to wildfires through the emergence of optimal habitat, populations eventually decline as the habitat changes to late succession (non-optimal forage) vegetation. During the 20 years following the last major fire in GMU 15A (1969), the population has declined by approximately 40%. Current and previous assessment of calf survival from this area has identified low calf survival (Franzmann et al. 1980, T. J. McDonough, unpublished data). Such a drastic decline in population size coupled with low productivity can negatively impact genetic diversity of a population; this may partially explain the significantly low genetic diversity on the Kenai Peninsula. A reduction in genetic diversity can lower viability and fecundity (Falconer 1981, Ralls et al. 1983, Frankham 1995, Crnokrak and Roff 1999), and at the extreme can lead to inbreeding depression; decreased viability and fecundity occur currently on the Kenai Peninsula (Franzmann and Schwartz 1985, ADF&G 2013, unpublished data). Whether lower reproductive rates (twinning rates and calf survival) on the Kenai Peninsula are correlated solely with genetic variability or are influenced in addition, or solely by environmental factors, is an area for future investigation.

**Conservation implications**

Although the effects of inbreeding depression can diminish over time (Lynch 1977), a general loss of genetic diversity can be detrimental over evolutionary time as it may lower the ability of populations to respond to environmental stressors such as novel predators, parasites, or climatic conditions (Lacy 1987, Quattro and Vrijenhoek 1989, Leberg 1993). Following the recommendations of Frankham et al. (2014), all 3 populations fall below the minimum effective population size of 1,000 required to maintain long-term viability. In addition, the GMUs on the Kenai Peninsula, when considered separately, have an effective population size lower than both recent (> 100; Frankham et al. 2014) and earlier (> 50; Franklin 1980, Soulé 1980) recommendations to avoid inbreeding depression. Indeed, the Kenai Peninsula does have a higher inbreeding coefficient (although not significantly different from zero) and higher levels of homozygosity. However, when considering the Kenai Peninsula as a single population, the effective population exceeds 100 but remains below the threshold for long-term viability.

Neutral loci are commonly used to infer evolutionary history of populations and make inferences about overall variation (see Howes and Lougheed 2008), but it is still unclear whether the trends in putatively neutral loci are reflective of quantitative-trait variation found in genes for physiological, morphological, or life history traits that are likely important for the adaptive potential of populations (Merilä and Crnokrak 2001, Reed and Frankham 2001, Eckert et al. 2008). Therefore, a conclusion that reduced genetic diversity observed at neutral microsatellite markers reflects reduction of diversity in the genome overall is premature. Our results showing significant population structure and limited connectivity to outside populations for the Kenai Peninsula provide a working hypothesis for the potential effects on genetic diversity, which can be tested by assaying both selectively neutral and functional diversity. Such studies can provide greater resolution on the processes responsible for the distribution of genetic diversity among moose populations within south-central Alaska.

## ACKNOWLEDGEMENTS

Funding was provided by Joint Base Elmendorf-Richardson, under the guidance of H. Griese, C. Garner, D. Battle, and R. Graham, and U. S. Geological Survey. Extensive field assistance was provided by the military conservation agent program on Elmendorf Air base. J. Crouse, J. Selinger, and pilots J. DeCreft, T. Levanger, and J. Fieldman, and the Kenai National Wildlife Refuge provided field assistance on the Kenai Peninsula. Z. Grauvogel, K. Sage, and S. Sonsthagen provided laboratory and manuscript advice, while K. Hundertmark provided advice on marker selection and G. Roffler and E. Solomon provided help with maps. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

## REFERENCES

Akerman, A., and R. Bürger. 2014. The consequences of gene flow for local adaptation and differentiation: a two-locus deme model. Journal of Mathematical Biology 68: 1135–1198.

Beaumont, M. A., W. Zhang, and D. J. Balding. 2002. Approximate Bayesian computation in population genetics. Genetics 162: 2025–2035.

Beerli, P., and J. Felsenstein. 1999. Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. Genetics 152: 763–773.

———, and ———. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proceedings of the National Academy of Sciences USA 98: 4563–4568.

Bishop, M. D., S. M. Kappes, J. W. Keele, R. T. Stone, S. L. F. Sunden, G. A. Hawkins, S. S. Toldo, R. Fries, M. D. Grosz, J. Yoo, and C. W. Beattie. 1994. A genetic linkage map for cattle. Genetics 136: 619–639.

Blows, M. W., and A. A. Hoffmann. 2005. A reassessment of genetic limits to evolutionary change. Ecology 86: 1371–1384.

Bourne, E. C., G. Bocedi, J. M. J. Travis, R. J. Pakeman, R. W. Brooker, and K. Schiffers. 2014. Between migration load and evolutionary rescue: dispersal, adaptation and the response of spatially structured populations to environmental change. Proceedings of Royal Society B 281: 20132795.

Bradshaw, A. D. 1991. The Croonian Lecture, 1991: genostasis and the limits to evolution. Philosophical Transactions of the Royal Society B: Biological Sciences 333: 289–305.

Briggs, J. C. 1996. Biogeography and punctuated equilibrium. Biogeographica 72: 151–156.

Brown, J. H., D. W. Mehlman, and G. C. Stevens. 1995. Spatial variation in abundance. Ecology 76: 2028–2043.

Coltman, D. W., J. G. Pilkington, J. A. Smith, and J. M. Pemberton. 1999. Parasite-mediated selection against inbred Soay sheep in a free-living, island population. Evolution 53: 1259–1267.

Coulson, T., S. Albon, J. Slate, and J. Pemberton. 1999. Microsatellite loci reveal sex-dependent responses to inbreeding and outbreeding in red deer calves. Evolution 53: 1951–1960.

Cornuet, J. M., and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144: 2001–2014.

Crnokrak, P., and D. A. Roff. 1999. Inbreeding depression in the wild. Heredity 83: 260–270.

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.

Eckert, C. G., K. E. Samis, and S. C. Lougheed. 2008. Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. Molecular Ecology 17: 1170–1188.

Ellegren, H. 2004. Microsatellites: simple sequences with complex evolution. Nature Reviews Genetics 5: 435–445.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software Structure: a simulation study. Molecular Ecology 14: 2611–2620.

Excoffier, L., and H. E. L. Lischer. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10: 564–567.

Falconer, D. S. 1981. An Introduction to Quantitative Genetics. Longman, London, England.

Farley, S., P. Barboza, H. Griese, and C. Gardner. 2012. Characterization of moose movement patterns, movement of black bears in relation to anthropogenic food sources, and wolf distribution and movement on JBER lands, of Elmendorf AFB and Fort Richardson AP. Alaska Department of Fish and Game Report, Juneau, Alaska, USA.

Franklin, I. R. 1980. Evolutionary change in small populations. Pages 135–149 *in* M. E. Soulé and B. A. Wilcox, editors. Conservation Biology: An Evolutionary-Ecological Perspective. Sinauer Associates, Sunderland, Massachusetts, USA.

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

———. 2015. Genetic rescues of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow. Molecular Ecology doi: http://10.1111/mec.13139.

———, C. J. A. Bradshaw, and B. W. Brook. 2014. Genetics in conservation management: revised recommendations for the 50/500 rules, red list criteria and population viability analyses. Biological Conservation 170: 56–63.

Franzmann, A.W., and P. D. Arneson. 1973. Moose Research Center Studies. Alaska Department of Fish and Game Report, Soldotna, Alaska, USA.

———, and C. C. Schwartz. 1985. Moose twinning rates: a possible population condition assessment. Journal of Wildlife Management 49: 394–396.

———, ———, and R. O. Peterson. 1980. Moose calf mortality in summer on the Kenai Peninsula, Alaska. Journal of Wildlife Management 44: 764–768.

Gaines, M. S., J. E. Diffendorfer, R. H. Tamarin, and T. S. Whittam. 1997. The

effects of habitat fragmentation on the genetic structure of small mammal populations. Journal of Heredity 88: 294–304.

García-Ramos, G., and M. KirkPatrick. 1997. Genetic models of adaptation and gene flow in peripheral populations. Evolution 51: 21–28.

Garza, J. C., and E. G. Williamson. 2001. Detection of reduction in population size using data from microsatellite loci. Molecular Ecology 10: 305–318.

Goudet, J. 1995. FSTAT (version 1.2): a computer program to calculate F-statistics. Journal of Heredity 86: 485–486.

Hampe, A., and R. J. Petit. 2005. Conserving biodiversity under climate change: the rear edge matters. Ecology Letters 8: 461–467.

Hartl, D. L., and A. G. Clark. 2007. Principle of Population Genetics, 4th Edition. Sinauer Associates, Sunderland, Massachusetts, USA.

Hedrick, P. W. 2005. A standardized genetic differentiation measure. Evolution 59: 1633–1638.

Hoffmann, A. A., and M. W. Blows. 1994. Species borders: ecological and evolutionary perspectives. Trends in Ecology and Evolution 9: 223–227.

———, and P. A. Parsons. 1991. Evolutionary Genetics and Environmental Stress. Oxford University Press, Oxford, England.

Howes, B. J., and S. C. Lougheed. 2008. Genetic diversity across the range of temperate lizard. Journal of Biogeography 35: 1269–1278.

Hubisz, M. J., D. Falush, M. Stephens, and J. K. Pritchard. 2009. Inferring weak population structure with the assistance of sample group information. Molecular Ecology Resources 9: 1322–1332.

Hughes, A. L. 2010. Reduced microsatellite heterozygosity in island endemics supports the role of long-term effective population size in avian microsatellite diversity. Genetica 138: 1271–1276.

Hundertmark, K. 2009. Reduced genetic diversity in two introduced and isolated moose populations in Alaska. Alces 45: 137–142.

Klein, D. R. 1970. Food selection by North American deer and their response to over-utilization of preferred plant species. Pages 25–46 *in* A. Watson, editor. Animal Populations in Relation to Their Food Sources. British Ecological Society Symposium 10. Blackwell, Oxford, England.

Lacy, R. C. 1987. Loss of genetic diversity from managed populations: Interacting effects of drift, mutation, immigration, selection, and population subdivision. Conservation Biology 1: 143–158.

Leberg, P. 1993. Strategies for population reintroduction: effects of genetic variability on population growth and size. Conservation Biology 7: 194–199.

Loranger, A. J., T. N. Bailey, and W. W. Larned. 1991. Effects of forest succession after fire in moose wintering habitats on the Kenai Peninsula, Alaska. Alces 27: 100–109.

Lynch, C. B. 1977. Inbreeding effects upon animals derived from a wild population of *Mus musculus*. Evolution 31: 526–537.

Mainguy, J., S. D. Côté, and D. W. Coltman. 2009. Multilocus heterozygosity, parental relatedness and individual fitness components in a wild mountain goat, *Oreamnus americanus* population. Molecular Ecology 18: 2297–2306.

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

Medrano, J. F., E. Aasen, and L. Sharrow. 1990. DNA extraction from nucleated red blood cells. Biotechniques 8: 43.

Meirmans, P. G. 2006. Using the AMOVA framework to estimate a standardized

genetic differentiation measure. Evolution 60: 2399–2402.

Merilä, J., and P. Crnokrak. 2001. Comparison of genetic differentiation at marker loci and quantitative traits. Journal of Evolutionary Biology 14: 892–903.

Miller, M. J., E. Bermingham, J. Klicka, P. Escalante, and K. Winker. 2010. Neotropical birds show a humped distribution of within-population genetic diversity along a latitudinal transect. Ecological Letters 13: 576–586.

Morton, J. M., M. Bower, E. Berg, D. Magness, and T. Eskelin. 2009. Long Term Ecological Monitoring Program on the Kenai National Wildlife Refuge, Alaska: an FIA adjunct inventory. Pages 1–17 *in* W. McWilliams, G. Moisen, and R. Czapiewski, compilers. Proceedings of the Forest Inventory and Analysis (FIA) Symposium 2008. RMRS- P-56CD. USDA Forest Service, Rocky Mountain Research Station, Fort Collins, Colorado, USA.

Muhs, D., T. A. Ager, and J. E. Beget. 2001. Vegetation and paleoclimate of the last interglacial period, central Alaska. Quaternary Science Reviews 20: 41–61.

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

Oldemeyer, J. L., A. W. Franzmann, A. L. Brundage, P. D. Arneson, and A. Flynn. 1977. Browse quality and the Kenai moose population. Journal of Wildlife Management 41: 533–542.

Parmesan, C., and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature 421: 37–42.

Patten, M. A., and C. L. Pruett. 2009. The song sparrow, *Melospiza melodia*, as a ring species: patterns of geographic variation, a revision of subspecies, and implications for speciation. Systematics and Biodiversity 7: 33–62.

Pielou, E. C. 1991. After the Ice Age: The Return of Life to Glaciated North America. University of Chicago Press, Chicago, Illinois, USA.

Piry, S., G. Luikart, and J. M. Cornuet. 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. Journal of Heredity 90: 502–503.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945–959.

Quattro, J. M., and R. C. Vrijenhoek. 1989. Fitness differences among remnant populations of the Sonoran topminnow, *Poeciliopsis occidentalis*. Science 245: 976–978.

Ralls, K., J. Ballou, and R. L. Brownell Jr. 1983. Genetic diversity in California sea otters: Theoretical considerations and management implications. Biological Conservation 25: 209–232.

Rambaut, A., and A. J. Drummond. 2007. Tracer v1.4. < http://beast.bio.ed.ac.uk/Tracer> (accessed August 2013).

Reed, D. H., and R. Frankham. 2001. How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. Evolution 55: 1095–1103.

———, and ———. 2003. Correlation between fitness and genetic diversity. Conservation Biology 17: 230–237.

Robinson, S. J., L. P. Waits, and I. D. Martin. 2007. Evaluating population structure of black bears on the Kenai Peninsula using mitochondrial and nuclear DNA analyses. Journal of Mammalogy 88: 1288–1299.

Røed, K. H., and L. Midthjell. 1998. Microsatellites in reindeer, *Rangifer tarandus*, and their use in other cervids. Molecular Ecology 7: 1773–1776.

Roffler, G. H., L. G. Adams, S. L. Talbot, G. K. Sage, and B. W. Dale. 2012. Range overlap and individual movements

during breeding season influence genetic relationships of caribou herds in south-central Alaska. Journal of Mammalogy 95: 1318–1330.

Root, T. L., J. T. Price, K. R. Hall, S. H. Schneider, C. Rosenzweig, and J. A. Pounds. 2003. Fingerprints of global warming on wild animals and plants. Nature 421: 57–60.

Schlötterer, C. 2000. Evolutionary dynamics of microsatellite DNA. Chromosoma 109: 365–371.

Schmidt, J. I., K. J. Hundertmark, R. T. Bowyer, and K. G. McCracken. 2009. Population structure and genetic diversity of moose in Alaska. Journal of Heredity 100: 170–180.

Schrey, A. W., M. Grispo, M. Awad, M. B. Cook, E. D. McCoy, H. R. Mushinsky, T. Albaryrak, S. Bensch, T. Burke, L. K. Butler, R. Dor, H. B. Fokidis, H. Jensen, T. Imboma, M. M. Kessler-Rios, A. Marzal, I. R. K. Stewart, H. Westerdahl, D. F. Westneat, P. Zehtindjiev, and L. B. Martin. 2011. Broad-scale latitudinal patterns of genetic diversity among native European and introduced house sparrow (*Passer domesticus*) populations. Molecular Ecology 20: 1133–1143.

Schwartz, C. C., and A. W. Franzmann. 1989. Bears, wolves, moose and forest succession, some management considerations on the Kenai Peninsula, Alaska. Alces 25: 1–10.

Schwartz, M. K., L. S. Mills, Y. Ortega, L. F. Ruggiero, and F. W. Allendorf. 2003. Landscape location effects genetic variation of Canada lynx (Lynx canadensis). Molecular Ecology 12: 1807–1816.

Silva, A. D., J.-M. Gaillard, N. G. Yoccoz, A. J. M. Hewison, M. Galan, T. Coulson, D. Allaine, L. Vial, D. Delorme, G. Van Laere, F. Klein, and G. Luikart. 2009. Heterozygosity-fitness correlations revealed by neutral and candidate gene markers in roe deer from a long-term study. Evolution 63: 403–417.

Sonsthagen, S. A., S. L. Talbot, and C. M. White. 2004. Gene flow and genetic characterization of Northern Goshawks breeding in Utah. Condor 106: 826–836.

Soulé, M. E. 1980. Thresholds for survival: maintaining fitness and evolutionary potential. Pages 151–169 *in* M. E. Soulé and B. A. Wilcox, editors. Conservation Biology: An Evolutionary-Ecological Perspective. Sinauer Associates, Sunderland, Massachusetts, USA.

Tallmon, D. A., A. Koyuk, G. H. Luikart, and M. A. Beaumont. 2008. ONeSAMP: a program to estimate effective population size using approximate Bayesian computation. Molecular Ecology Resources 8: 299–301.

Testa, J. W., and G. P. Adams. 1989. Body condition and adjustments to reproductive effort in female moose (*Alces alces*). Journal of Mammalogy 79: 1345–1354.

Tomasik, E., and J. A. Cook. 2005. Mitochondrial phylogeography and conservation genetics of wolverine (*Gulo gulo*) of northwestern North America. Journal of Mammalogy 86: 386–396.

Von Hardenberg, A., B. Bassano, M. Festa-Bianchet, G. Luikart, P. Lanfranchi, and D. Coltman. 2007. Age-dependent genetic effects on a secondary sexual trait in male alpine ibex, *Capra ibex*. Molecular Ecology 16: 1969–1980.

Vucetich, J. A., and T. A. Waite. 2003. Spatial patterns of demography and genetic processes across the species' range: null hypotheses for landscape conservation genetics. Conservation Genetics 4: 639–645.

Wilson, G. A., and B. Rannala. 2003. Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163: 1177–1191.

———, C. Strobeck, L. Wu, and J. W. Coffin. 1997. Characterization of microsatellite loci in caribou *Rangifer tarandus*, and their use in other artiodactyls. Molecular Ecology 6: 697–699.

WISELY, S. M., S. W. BUSKIRK, G. A. RUSSELL, K. B. AUBRY, and W. J. ZIELINKSI. 2004. Genetic diversity and structure of the fisher (*Martes pennanti*) in a peninsular and peripheral metapopulation. Journal of Mammaology 85: 640–648.

YAMASHITA, T., and G. A. POLIS. 1995. A test of the central-marginal model using sand scorpion populations (*Paruroctonus mesaensis*, Vaejovidae). Journal of Arachnology 23: 60–64.