Conservation Genetics (2023) 24:235–248
https://doi.org/10.1007/s10592-022-01496-w

RESEARCH ARTICLE

Genetic diversity and connectivity of moose (*Alces americanus americanus*) in eastern North America

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Received: 20 June 2022 / Accepted: 11 December 2022 / Published online: 16 January 2023

Abstract

Genetic diversity is critical to a population's ability to overcome gradual environment change. Large-bodied wildlife existing in regions with relatively high human population density are vulnerable to isolation-induced genetic drift, population bottlenecks, and loss of genetic diversity. Moose (*Alces americanus americanus*) in eastern North America have a complex history of drastic population changes. Current and potential threats to moose populations in this region could be exacerbated by loss of genetic diversity and connectivity among subpopulations. Existing genetic diversity, gene flow, and population clustering and fragmentation of eastern North American moose are not well quantified, while physical and anthropogenic barriers to population connectivity already exist. Here, single nucleotide polymorphism (SNP) genotyping of 507 moose spanning five northeastern U.S. states and one southeastern Canadian province indicated low diversity, with a high proportion of the genomes sharing identity-by-state, with no consistent evidence of non-random mating. Gene flow estimates indicated bidirectionality between all pairs of sampled areas, with magnitudes reflecting clustering and differentiation patterns. A Discriminant Analysis of Principal Components analysis indicated that these genotypic data were best described with four clusters and indicated connectivity across the Saint Lawrence River and Seaway, a potential physical barrier to gene flow. Tests for genetic differentiation indicated restricted gene flow between populations across the Saint Lawrence River and Seaway, and between many sampled areas facing expanding human activity. These results document current genetic variation and connectivity of moose populations in eastern North America, highlight potential challenges to current population connectivity, and identify areas for future research and conservation.

Keywords Moose · Population genetics · Connectivity · Gene flow · Inbreeding · Genetic diversity

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Introduction

Genetic diversity is an important factor for the persistence of wildlife populations (Frankham 2005; Allendorf et al. 2010). Maintaining genetic diversity can improve population resilience in the face of unknown future conditions, reducing extinction risk and increasing population stability (Forsman and Wennersten 2016). However, many wildlife populations have been negatively impacted by anthropogenic activities such as habitat alterations, overhunting, and climate change (Ripple et al. 2015; Vitousek et al. 1997; Allendorf et al. 2008; Laikre et al. 2010b). Reductions of wildlife populations, together with the loss of connectivity between populations, can reduce genetic diversity and effective population size, gene flow, and a population's resilience to new challenges (Reed and Frankham 2003).

Large-bodied species are particularly vulnerable to the effects of landscape change because they occur in low density, have large home ranges and low fecundity, and relatively long development times (Ripple et al. 2015). These characteristics also make large-bodied wildlife genetically vulnerable to the long-lasting effects of their population's founding, bottlenecks, and isolation. The moose (*Alces alces*) in North America is a species with a complex history of expansions and declines, driven by environmental and anthropogenic pressures that have impacted the species' genetic diversity (Broders et al. 1999; Hundertmark et al. 1993; Hundertmark et al. 2002). Moose arrived relatively recently in North America (within the past 15,000 years) by crossing the Bering land bridge from Asia to Alaska and the Yukon Region (Hundertmark et al. 2002; Hundertmark et al. 2003). Moose expanded their range as glaciers receded and quality forage became available (mainly within the past 8,000 years), with subsequent peripheral expansions by small groups of animals (Hundertmark et al. 2003). Moose continued to radiate eastwards through what is now the midwestern United States (Dyke and Prest 1987) and established populations in eastern North America (Hundertmark et al. 2003). This eastern segment became isolated by a warming climate, resulting in the eastern moose subspecies present today that occurs in the northeastern United States and Canada (*A. a. americana;* Hundertmark et al. 2003).

The eastern subspecies of moose has undergone dramatic population fluctuations over the past two centuries, which probably exacerbated the founder effects from the establishment of populations (Kalbfleisch et al. 2018). Moose in the northeastern United States experienced severe population declines during the 19th century with land conversion to agriculture and unregulated hunting (Goodwin 1936; Wattles and DeStafeno 2011). Hunting regulations and reforestation in the 20th century allowed for the recolonization of moose to much of the region from populations in the state of Maine and the southern region of the province of Quebec (Wattles and DeStafeno 2011). The region had recovering moose populations by the 1970s and 1980s (Alexander 1993; Bontaites & Gustafson 1993; Morris and Elowe 1993; Musante et al. 2010), with populations reaching the highest densities on record in the early 2000s.

Moose population densities in eastern North America in the past two decades have declined in the face of substantial impacts from winter ticks (*Dermacentor albipictus*) that have caused epizootics in populations in the northeastern United States (Bergeron et al. 2013; Jones et al. 2017; DeBow et al. 2021). The physiological impact of high tick infestation increases juvenile mortality rates and reduces per capita birth rates, contributing to recent population declines in the northeastern United States (Musante et al. 2007, 2010; Bergeron et al. 2013; Timmermann & Rodgers 2017; Ellingwood et al. 2020; DeBow et al. 2021; Rosenblatt et al. 2021). Winter tick epizootics are expected to continue and potentially increase in frequency with climate change (Dunfey-Ball 2017). More broadly, climate change is expected to bring an assortment of growing challenges for moose, including reduced habitat availability and increased disease exposure (Murray et al. 2006; Lankester et al. 2010; Ellingwood et al. 2020; Pearman-Gillman et al. 2020a, b; DeBow et al. 2021; Blouin et al. 2021a, b).

The combined history of moose in North America, recent population declines, and the continued threat from winter tick infestation expected with future climate conditions highlight a need for understanding the genetic variation and connectivity of moose populations in this region in eastern North America. Little is known of the adaptive capacity of moose populations in this region to persist in challenging conditions, and the importance of genetic variation in populations' abilities to persist in adverse conditions. To begin to answer broader questions around adaptive capacity, simple analyses can build a better understanding of current genetic variation and connectivity in the region. First, estimating genetic diversity and inbreeding with common metrics such as observed heterozygosity and inbreeding coefficients will aid in our understanding of the echoes of the radiation of moose to eastern North America and more recent population dynamics that can influence adaptive capacity. Across their distribution in North America, moose populations exhibit low genetic diversity, decreasing in eastern populations (Cronin et al. 2001; Ferrante et al. 2021). Basic information regarding regional genetic diversity and inbreeding are not well quantified in eastern North America, where a distinct subspecies of moose faces its greatest challenge. Second, estimating gene flow between populations could provide insights into the maintenance of current genetic diversity through the exchange of genetic material. In eastern North America potential barriers to gene flow, such as the Saint

Lawrence River and Seaway (SLRS) and expansive human development have remained understudied despite rapidly changing climate and anthropogenic conditions on the landscape. Finally, estimating population differentiation with common F_{st} metrics could identify populations that are increasingly isolated in eastern North America due to these potential barriers (Weir and Cockerham 1984). Elsewhere in higher latitudinal ranges with little human influence in Alaska, USA and northwestern Canada moose populations have minimal genetic differentiation over long distances despite topographic barriers (Schmidt et al. 2009). However, studies have documented signatures of isolation and genetic drift presumed to result from the radiation of moose across North America, followed by a complex history of human-driven extirpation and habitat alteration (Broders et al. 1999; Wilson et al. 2003; DeCesare et al. 2020). Filling these knowledge gaps in eastern North America is important for integrating aspects of population genetics with landscape protection, harvest management, and forecasting the challenging future for moose (Frankel 1974; Frankham 2005; Allendorf et al. 2010; Laikre et al. 2010a; Ferrante et al. 2021).

This study aimed to improve our understanding of the genetic diversity, genetic structure, and connectivity of moose in the eastern North America, which face a suite of increasing challenges. We used a recently developed panel of Single Nucleotide Polymorphism (SNP) markers (Kalbfleisch et al. 2018) to genotype moose across five states in the northeastern United States and the province of Quebec in Canada to (1) estimate levels of genetic diversity and inbreeding across the region, (2) estimate relative migration rates between sampled areas, and (3) document genetic structure and fragmentation using a cluster analysis and differentiation statistics. The results tested whether the SLRS acts as a barrier to gene flow between moose populations and help to strengthen our understanding of current genetic variability and connectivity of moose populations in eastern North America.

Methods

Study area and sample collection

Hair and tissue samples were collected from 529 moose across their regional distribution including nine sampled areas in five states in the northeastern United States (New York, Vermont, New Hampshire, Maine, and Massachusetts) and the province of Quebec in Canada (Fig. 1). Samples were collected in three distinct geographic areas of interest in Quebec including two in Quebec province north of the SLRS (western Quebec and northeastern Quebec), and one sampled area south of the SLRS (southeastern Quebec). Samples were collected from two targeted areas in Maine (northern and western Maine) that represent the likely source of moose recolonizing much of the region in the 20th century (Wattles and DeStafeno 2011). Hair samples were collected in discrete areas during radio-collar studies in Vermont under the auspices of University of Vermont IACUC protocol #17–035 (n = 106; 2016–2019; DeBow et al. 2021; Blouin et al. 2021 a,b; Rosenblatt et al. 2021), New Hampshire (n = 34; 2015–2017; Jones 2017; Ellingwood et al. 2020), and Maine (n = 57; 2015–2017). Hair and tissue samples were opportunistically collected from animals that were harvested, died in vehicle collisions, or translocated throughout Vermont (n = 105; 2014–2017), Quebec (n = 198; 2019), Massachusetts (n = 5; 2018–2019), and New York (n = 24; 2011–2016). Sampled moose varied in age class (juvenile, adult) and included both sexes, with some known parent-offspring pairs from radio-collared individuals.

Sample processing and genotyping

Hair samples from moose across the sampled areas were stored at room temperature or -20˚C after collection. Muscle samples were either stored at -20 ˚C or desiccated after collection. DNA was extracted from all samples using a phenol-chloroform extraction process as previously described (Sambrook et al. 1989). Of the 317 autosomal moose SNPs identified by Kalbfleisch et al. (2018) as highly conserved, 136 loci were utilized to develop a MALDI-TOF MS genotyping assay as described in Heaton et al. (2014). Assay development and genotyping were performed by Neogen (Lincoln, Nebraska, USA). Quality control of genotype data was performed using dartR (Gruber et al. 2018) in R (R Core Team 2021) to filter (1) loci with a call rate less than 90%, (2) individuals with less than 90% of loci genotyped, and (3) loci that had a minor allele frequency < 5%. The hw.test() function from the pegas package (Paradis 2010) was used to test if any loci consistently were out of Hardy-Weinberg Equilibrium (HWE) across all nine sampled areas. After filtering problematic loci and individuals, genotypes from 112 to 136 SNPs (82%) were obtained for 507 individuals and all loci met Hardy-Weinberg expectations in the nine geographic regions samples. We analyzed these genetic data with the three objectives described below. All data presented and analyzed in this manuscript can be found in the USGS ScienceBase Repository (McKay et al. 2022).

Objective 1: genetic diversity

Each population's genetic diversity was described using rarefied allele richness (AR, ranging from 1 to 2 for SNP loci) and expected and observed heterozygosity (He & Ho,

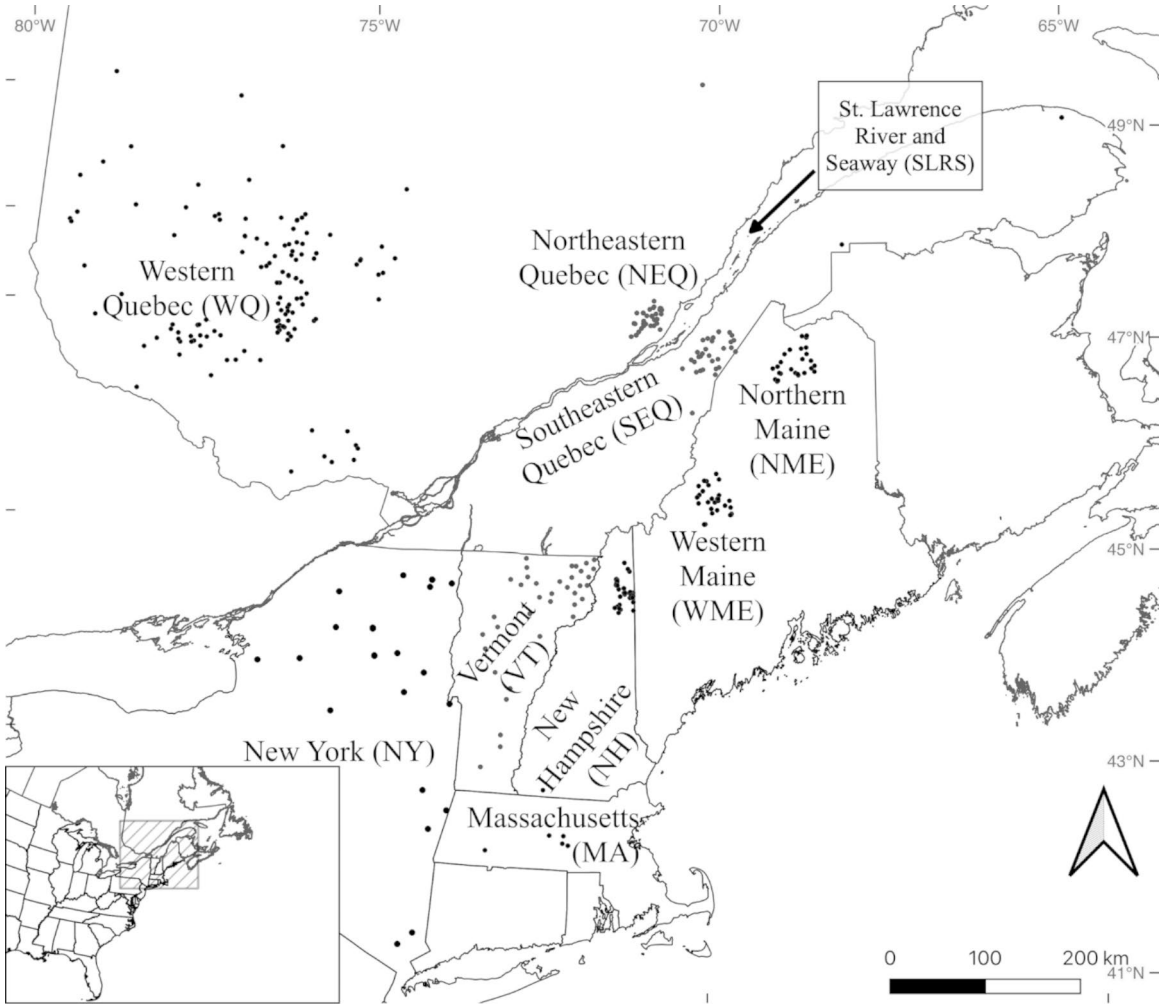

Fig. 1 The distribution of 529 moose (*Alces americanus americanus*) sampled (points) for this study across five states in the United States and one Canadian province. Text labels indicate the nine sampled areas targeted in this study. The Saint Lawrence River and Seaway (SLRS) is a potential barrier to gene flow in this region

ranging from 0 to 1) using the divBasic()_function from the diveRsity package in R (Hughes et al. 2008; Keenan et al. 2013; Greenbaum et al. 2014; R Core Team 2021). In this study, AR represented a metric of adaptive capacity, and expected and observed heterozygosity represented a correlate of individual fitness (Greenbaum et al. 2014). Inbreeding coefficients (FIS; Wright 1969) were estimated for each sampled area using divBasic() function from the diveRsity package (Keenan et al. 2013). Confidence intervals were generated for allelic richness and inbreeding coefficient with 1,000 bootstraps to determine if AR differed between sampled areas and if FIS differed from 0, which would indicate inbreeding or outbreeding. Finally, we calculated identity by state (IBS) probabilities between all individuals using the SNPRelate package (Zheng et al. 2012) to estimate the proportion of SNP alleles shared between individuals. We reported the average IBS proportions within and among sampled areas.

Objective 2: regional gene flow

Gene flow between sampled areas was estimated using the divMigrate() function in the diveRsity package (Keenan et al. 2013). The divMigrate() function with "Nm" selected as the calculated statistic produced the estimated number of migrants per generation moving between all pairs of sampled areas, derived from genetic differentiation between populations (Sundqvist et al. 2016). The resulting estimates of gene flow were scaled by the divMigrate() function based on the highest number of migrants per generation, providing relative rates of gene flow ranging from 0 to 1. Any source-sink dynamics between pairs of sampled areas were identified if 95% confidence intervals around relative gene flow rates did not overlap. These confidence intervals were generated with the divMigrate() function over 4,999 bootstraps. We plotted relative gene flow rates between sampled areas provided by the divMigrate() function, to illustrate the

Table 1 Average genetic diversity and inbreeding (FIS) measures for moose (*Alces americanus americanus*) across the nine sampled areas. We quantified genetic diversity with measures of allelic richness, observed heterozygosity (Ho) and expected heterozygosity (He)

Sampled Area	Sample Size	Allelic Richness (95% CI)	H_o	H_e	FIS (95% CI)
Northeastern Quebec (NEQ)	38	1.78 (1.71–1.84)	0.3328	0.3309	-0.006 (-0.047–0.034)
Southeastern Quebec (SEQ)	34	1.86 (1.79–1.91)	0.3585	0.3593	0.002 (-0.040-0.044)
Massachusetts (MA)	5	1.79 (1.63–1.88)	0.3571	0.3254	-0.098 (-0.346–0.010)
Northern Maine (NME)	28	1.86 (1.78–1.93)	0.3572	0.3549	-0.007 (-0.055–0.038)
Western Maine (WME)	26	1.87 (1.79–1.93)	0.3537	0.3641	0.028 (-0.014–0.065)
New Hampshire (NH)	30	1.85 (1.77–1.90)	0.3518	0.3500	-0.005 (-0.047–0.031)
New York (NY)	23	1.88 (1.79–1.94)	0.3594	0.3735	0.038 (-0.022–0.091)
Vermont (VT)	203	1.87 (1.81–1.92)	0.3632	0.3646	0.004 (-0.011–0.019)
Western Quebec (WQ)	120	1.87 (1.81–1.91)	0.3582	0.3696	0.031 (0.012–0.050)

magnitude of relative migration rates and the clustering of similar sampled areas (Keenan et al. 2013).

Objective 3: regional genetic structure

We analyzed genetic structuring and clustering across sampled areas to better understand the level of differentiation across the region. First, Weir and Cockerham's fixation index (Fst) was calculated between all pairs of sampled areas using the genet.dist() function in the heirfstat package (Weir and Cockerham 1984; Goudet and Jombart 2020). We tested whether these F_{st} values were greater than 0, which would indicate genetic differentiation between subpopulations, using the boot.ppfst() function with 1,000 bootstraps to generate 95% confidence intervals.

We then identified the optimal number of clusters that best summarize the genetic variation captured across the region using the find.cluster() function in the adegenet package (Jombart 2008; Jombart and Ahmed 2011). This analysis used 120 principal components and considered clusters (k) ranging from 1 (a single, panmictic cluster) – 9 (each sampled area represented a unique cluster). Though it was unlikely that each sampled area represented distinct clusters, the cluster analysis was intentionally not constrained by a priori assumptions of how these sampled areas were clustered. The cluster analysis used the "kmeans" method and "diffNgroup" criterion to estimate differences in Bayesian Information Criterion (BIC; Schwartz 1978) between successive numbers of clusters to identify the most parsimonious number of clusters best describing the observed data (Jombart et al. 2010). The find.cluster() function generated assignment probabilities for each sampled individual to the resulting clusters (Beugin et al. 2018).

We then conducted a Discriminant Analysis of Principal Components (DAPC) to visualize differences between clusters using the adegenet package (Jombart 2008; Jombart and Ahmed 2011). Following Millet et al. (2020), an initial DAPC was run using the dapc() function, considering the cluster assignments described above with 120 principal components (n.pca) and 3 axes. (n.da). The optimal number of principal components was determined from the initial DAPC analysis using the optim.a.score() function to maximize the variability explained without risking overfitting (Jombart et al. 2010). A final DAPC was run with the optimal number of principal components for interpretation. An Analysis of Molecular Variance (AMOVA) was run using these cluster assignments and the poppr.amova() function from the poppr package (Kamvar et al. 2014, 2015) and the randtest() permutation function from the ade4 package (Chessel et al. 2004; Dray and Dufour 2007; Dray et al.,. 2007; Bougeard and Dray 2018; Thioulouse et al. 2018) to test the significance of variance explained within and between individuals and clusters.

Results

Objective 1: genetic diversity

Measures of genetic diversity indicated similar, low diversity across all sampled areas (Table 1; Fig. 2). Allelic richness ranged from 1.78 to 1.88, with allelic richness lowest in northeastern Quebec and in Massachusetts. However, 95% confidence intervals for allelic richness overlapped across all sampled areas. Observed and expected heterozygosity remained low across sampled areas, ranging from 0.3328 to

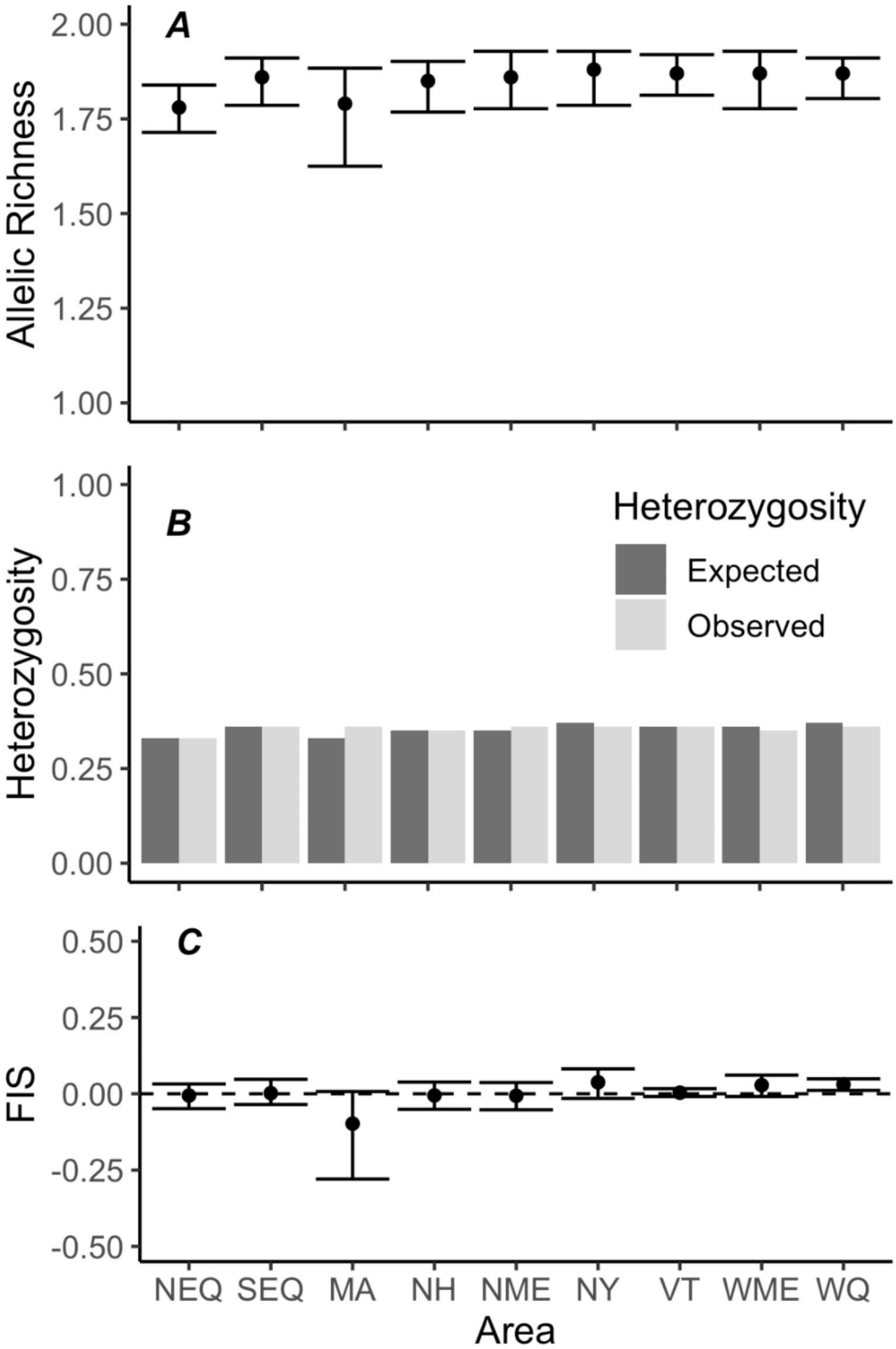

Fig. 2 Average genetic diversity and inbreeding (FIS) measures from moose (*Alces americanus americanus*) sampled across the nine areas. We quantified genetic diversity with measures of (A) allelic richness, (B) observed heterozygosity (Ho) and expected heterozygosity (He), and (C) inbreeding coefficients. Sampled areas included Northeastern Quebec (NEQ), Southeastern Quebec (SEQ), Massachusetts (MA), northern Maine (NME), western Maine (WME), New Hampshire (NH), Vermont, (VT), New York (NY), and Western Quebec (WQ)

0.3632 and 0.3254–0.3735, respectively. Average inbreeding coefficients (FIS) ranged from − 0.098 to 0.038 across sampled areas. In all areas except western Quebec, confidence intervals around FIS estimates did not detectably differ from 0, indicating a lack of evidence of inbreeding or outbreeding. Inbreeding coefficients for moose in western Quebec indicated low, but detectable levels of inbreeding (Table 1; Fig. 2). Identity-by-state (IBS) proportions were high within and among sampled areas, with an average of 69–73% of an individual's genotyped loci identical to another individual from the same population (Table S1).

Table 2 Pairwise relative gene flow rates for the nine areas where moose (*Alces americanus americanus*) were sampled, where row names indicate source populations and column names indicate recipient populations. Scale bars are added below values to illustrate differences in relative gene flow (ranging from 0–1). Abbreviations are defined in the caption of Fig. 2

Source population \ Recipient population	EQN	EQS	NH	NME	NY	VT	WME	WQ
EQN	-	0.09	0.07	0.09	0.10	0.08	0.08	0.20
EQS	0.06	-	0.39	0.63	0.44	0.64	0.65	0.12
NH	0.05	0.42	-	0.36	0.30	0.98	0.57	0.09
NME	0.05	0.65	0.32	-	0.31	0.48	0.48	0.10
NY	0.06	0.39	0.25	0.28	-	0.40	0.43	0.13
VT	0.05	0.64	0.88	0.45	0.44	-	1.00	0.10
WME	0.05	0.59	0.47	0.49	0.44	0.98	-	0.11
WQ	0.14	0.13	0.08	0.11	0.16	0.11	0.11	-

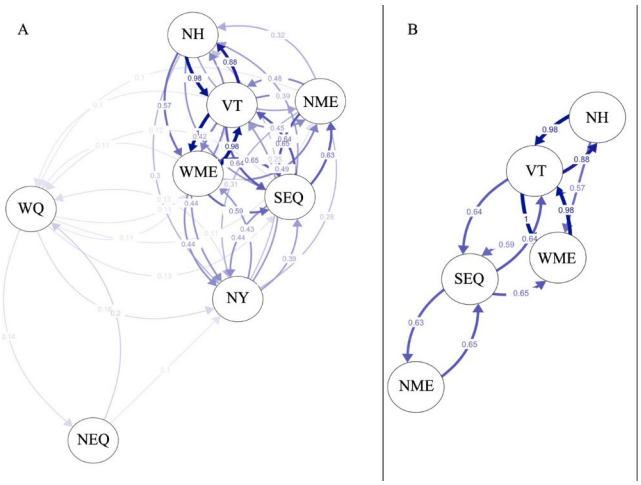

Fig. 3 Gene flow results for moose (*Alces americanus americanus*) using diveRsity package, where rates (0–1) represent relative values of number of migrants per generation (nM; Table 2). Sampled areas are encoded in circles and connected with arrows that darken and increase in size as gene flow rates increase. Abbreviations are defined in the caption of Fig. 2. Massachusetts (MA) samples were not included in this figure due to the small number collected. (A) After filtering the lowest 10% of gene flow rates, gene flow is greatest between sampled areas south of the Saint Lawrence River and Seaway (SLRS), with some connectivity to western Quebec (WQ) moose, sampled north of the SLRS. (B) When considering the top 50% of relative gene flow rates, there was high, bidirectional gene flow between sampled areas in southeastern Quebec (SEQ), Maine (NME and WME), New Hampshire (NH), and Vermont (VT)

Objective 2: regional gene flow

Relative gene flow estimation indicated various paths of genetic material across our study area (Table 2; Fig. 3). We did not detect any significant directional gene flow between any pair of sampled areas. Sampled areas south of the SLRS had relatively higher gene flow compared to sampled areas north of the SLRS (Fig. 3; Table 2). We estimated the highest relative rates of gene flow between Vermont and western Maine (0.98 and 1.00), and between Vermont and New Hampshire. Gene flow rates indicated that southeastern Quebec was most connected to northern Maine, western Maine, and Vermont (0.59–0.65). Outlying populations in New York and Massachusetts had lower relative gene flow to and from other sampled areas south of the SLRS (0.07–0.44 and 0.07–0.19, respectively).

North of the SLRS gene flow was somewhat higher between western Quebec and northeastern Quebec (0.14–0.20) compared to lower gene flow across the SLRS (0.04–0.13; Table 2). We estimated greater gene flow from sampled areas south of the SLRS to Western Quebec, compared to northeastern Quebec (0.08–0.13 and 0.04–0.06, respectively). We also estimated higher gene flow to sampled areas south of the SLRS from western Quebec compared to northeastern Quebec (0.04–0.16 and 0.03–0.10, respectively; Table 2). This exchange across the SLRS was greatest between Western Quebec and New York (Table 2).

Table 3 Pairwise genetic differentiation (Fst) between areas where moose (*Alces americanus americanus*) were sampled. Bootstrapped 95% confidence intervals for several Fst values differed from 0 (in bold), indicating genetic differentiation between the corresponding pair of sample areas

Sampled Area	NEQ	SEQ	MA	NME	WME	NH	NY	VT
Northeastern Quebec (NEQ)	-	-	-	-	-	-	-	-
Southeastern Quebec (SEQ)	**0.115**	-	-	-	-	-	-	-
Massachusetts (MA)	**0.142**	**0.018**	-	-	-	-	-	-
Northern Maine (NME)	**0.119**	0.001	**0.033**	-	-	-	-	-
Western Maine (WME)	**0.122**	0.001	0.006	0.004	-	-	-	-
New Hampshire (NH)	**0.142**	**0.012**	0.000	**0.016**	0.004	-	-	-
New York (NY)	**0.108**	**0.009**	**0.026**	**0.018**	0.005	**0.022**	-	-
Vermont (VT)	**0.127**	**0.010**	0.006	**0.014**	0.001	0.003	**0.016**	-
Western Quebec (WQ)	**0.053**	**0.073**	**0.096**	**0.081**	**0.079**	**0.098**	**0.061**	**0.089**

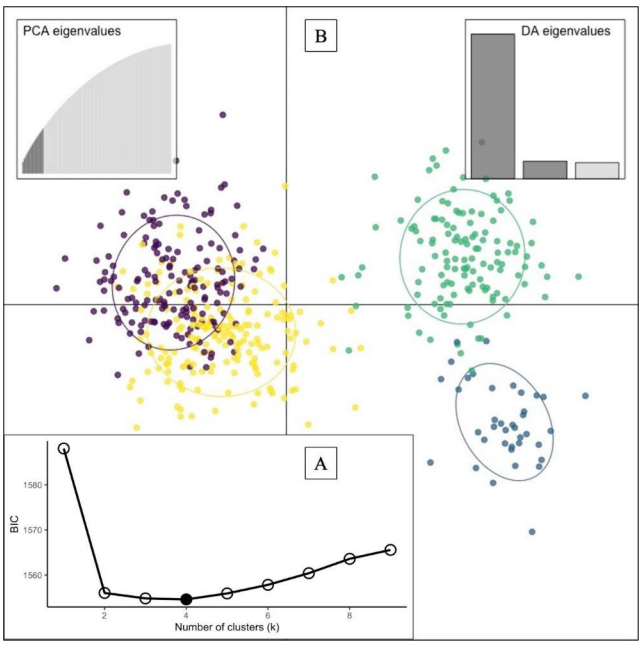

Fig. 4 Population clustering results indicated four distinct population clusters that best explain genetic variation across moose (*Alces americanus americanus*) sampled for this study. (A) Bayesian Information Criterion (BIC) scores generated with the find.cluster() function from the adegenet package indicated four clusters (k; black point) as the most parsimonious solution. (B) Visualization of the differences between these four clusters estimated by discriminant analysis of principal components (DAPC), with colored points indicating individuals assigned to each of the four clusters. This visualization was based on 16 principal components (PCA Eigenvalues) identified as the optimal number of components by the optim.a.score() function from the adegenet package (top left insert) and 3 discriminant analysis axes (top right insert)

Objective 3: regional genetic structure

Genetic differentiation was apparent across many sampled areas, despite the small geographic extent of the study (Table 3). Sampled areas north of the SLRS (northeastern Quebec and western Quebec) had significant pairwise F_{st} values with all sampled areas south of the SLRS (ranging from 0.108 to 0.142 and 0.061–0.098, respectively) and with each other (F_{st} = 0.053; Table 3). Sampled areas south of the SLRS had pairwise F_{st} values that were much lower (range: 0.0–0.033), but many of these pairwise F_{st} values did detectably differ from 0, thus indicating genetic differentiation among sampled areas. There was no evidence of differentiation between southeastern Quebec and both Maine sampled areas or between western Maine, New Hampshire, Vermont, and Massachusetts moose. F_{st} values for New York moose indicated small yet detectable differentiation from all other sampled areas south of the SLRS, except when compared to western Maine (Table 3). New York moose also had the lowest level of genetic differentiation with sampled areas north of the SLRS, compared to moose from other sampled areas south of the SLRS (Table 3).

The optimal number of clusters of moose that best summarized the genetic variation captured across the region was four distinct clusters (Fig. 4 A). Results from the DAPC analysis described genetic variability across three discriminant functions based on an optimal 16 principal components (Fig. 4B). The AMOVA revealed that 91.8% of genetic variation was contained within individuals, 1.5% among individuals within clusters, and 6.7% among clusters, with significant differences among clusters (p-value = 0.01; Table S2).

Individual moose posterior cluster membership probabilities estimated from the DAPC analysis indicated distinct clustering across the region (Fig. 5). North of the SLRS in western Quebec (WQ) and northeastern Quebec (NEQ) there were two distinct genetic clusters (blue and green columns in Fig. 5). WQ and NEQ sampled areas had their own principal cluster, with a few moose sampled in each area partially or completely assigned to the predominant cluster of the other (Fig. 5). Moose from south of the SLRS were mainly assigned to the two additional clusters (yellow and purple columns in Fig. 5). There was an apparent latitudinal shift in the principal cluster south of the SLRS. One cluster was more commonly assigned to southeastern Quebec and northern Maine moose (yellow columns; Fig. 5), while further south, cluster assignment was a mix of these two southern clusters. A few moose in southeastern Quebec, Northern Maine, and New York showed partial or

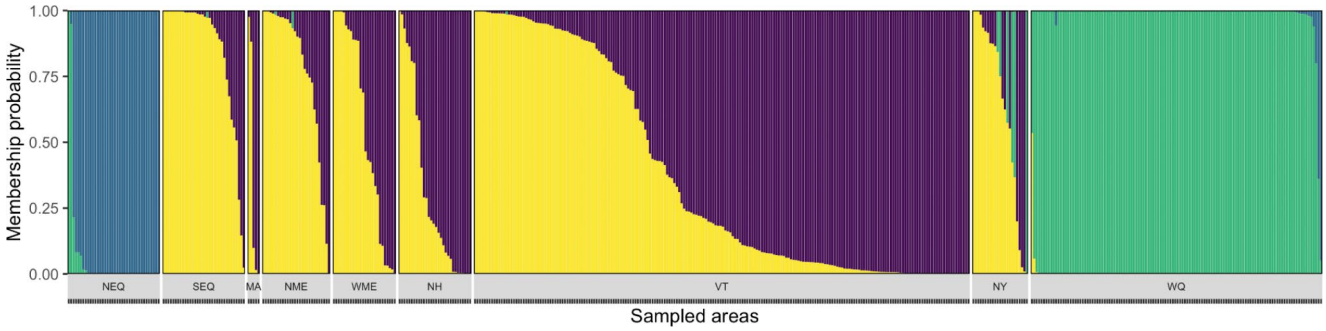

Fig. 5 Cluster assignment results for moose (*Alces americanus americanus*) from the nine sampled areas in this study, based on the four clusters identified in the cluster analysis. Each stacked bar indicates the mixture of assignment probabilities to each cluster (color) for each sampled individual. Sampled areas included Northeastern Quebec (NEQ), Southeastern Quebec (SEQ), Massachusetts (MA), northern Maine (NME), western Maine (WME), New Hampshire (NH), Vermont, (VT), New York (NY), and Western Quebec (WQ). NEQ and WQ sampled areas are north of the Saint Lawrence River and Seaway (SLRS); all other sampled areas are south of the SLRS (Fig. 1)

complete membership with the predominant cluster in western Quebec.

Discussion

Moose in eastern North America have a complex history of colonization, isolation, and bottlenecks (Broders et al. 1999; Hundertmark et al. 1993; Hundertmark et al. 2002; Hundertmark et al. 2003; Kalbfleisch et al. 2018). Climate change and future human development is expected to make much of the region less hospitable to moose (Pearman-Gillman et al. 2020a, b). The adaptive capacity of moose populations, particularly those that have limited connection to others, may play a role in their persistence into the future. The role of adaptive capacity and its interaction with evolving challenges across our study area lies beyond the inferences of this study. Our study is the first step in answering broader questions focused on the importance of this variation on the persistence of populations in the area by providing a current snapshot of genetic diversity, differentiation, and gene flow.

This study advances our understanding of moose population genetics in eastern North America with one of the largest genetic datasets generated for the North American moose. However, there are limitations to the dataset analyzed here. First, the number of genome-wide SNPs used in this study (112) is much smaller than recent genetic studies with 1920 SNPs identified from reduced representation restriction (RRR) libraries for genotyping by sequencing (GBS), albeit with a much smaller sample of 159 moose (Ferrante et al. 2021). The smaller SNP sample size may introduce bias in the results and may miss aspects of population structure (Moragues et al. 2010). However, we successfully genotyped a large sample size of 507 individuals, which aided in quantifying regional population structure and gene flow. Also, the reduced SNP density here was offset by the ease of access, analysis, and potential portability across other populations without need for making new RRR libraries for GBS. Additionally, the genome-wide SNP panel used here likely matches or exceeds the ability of the 8 microsatellite loci commonly genotyped in previous studies of moose population structure and differentiation (Broders et al. 1999; Kalinowski 2002). Second, sample collection was unbalanced across sampled areas due to the methods and distribution of sample collection and jurisdictional boundaries. These differences may bias or obscure some of our detected differentiation between sampled areas, particularly for areas with small sample sizes (Ruzzante 1998). For example, we include the five individuals from Massachusetts in our results, but acknowledge that we cannot interpret their role in structure, gene flow, and differentiation.

Diversity metrics indicated that while adaptive potential remains consistent across the areas we sampled (as measured by AR), sampled moose had low rates of heterozygosity and high proportions of their genotyped loci IBS. Our estimates of allelic richness and heterozygosity are comparable with previous studies of moose population genetics in eastern North America (Broders et al. 1999; Cronin et al. 2001; Ferrante et al. 2021). Further, observed heterozygosity from moose sampled in this study were lower than in previous studies of moose populations genetics in central and western North America (Table S3). We note that this comparison is nuanced given that our study used a newly developed SNP panel, and previous studies of genetic diversity in moose primarily relied on microsatellites (Zimmerman et al. 2020). These results generally are consistent with previously suggested pattern of reduced heterozygosity following the radiation of moose in North America (Kalbfleisch et al. 2018).

Current inbreeding coefficients (FIS) across sampled areas were very low, indicating that reduced heterozygosity was likely not due to non-random mating between related individuals. Our estimated FIS across the region was typical of moose populations elsewhere in North America including

Minnesota, Idaho, Montana, and Wyoming (Ferrante et al. 2021), in contrast with isolated moose populations in Michigan's Isle Royale experiencing decreasing heterozygosity and increasing inbreeding (Sattler et al. 2017). Nonetheless, high IBS values documented in this study indicated that moose shared similar genetic material. These contrasting results may be influenced by the unbalanced sampling in this study, but we note that this discrepancy existed in all sampled areas regardless of collection method and the number of moose sampled. Key to this contrast is the previously suggested idea that low levels of genetic variation in moose populations are the consequence of population bottlenecks (Cronin et al. 2001; Broders et al. 2002, Sattler et al. 2017). Low levels of heterozygosity may have resulted from genetic drift over the series of population founder events from the expansion of moose into eastern North America, and bottlenecks including the near extirpation of moose in the 19th century and recent population declines due to parasitic disease and associated epizootics. Through these founding and bottleneck events potential mates were increasingly likely to share a common ancestor, despite randomly mating, reducing effective population size. While we cannot rule out the contributions of random mutation in IBS, this explanation may result in the high IBS values and reduced heterozygosity.

There was no evidence that landscape fragmentation had isolated any sampled area in this study, according to our estimates of population differentiation and gene flow on either side of the SLRS. However, we report detectable differentiation with pair-wise F_{st} that is larger than differentiation reported in other studies of wild moose populations (Broders et al. 1999; Cronin et al. 2001; Schmidt et al. 2009). North of the SLRS, low but detectable levels of genetic differentiation existed between western Quebec and northeastern Quebec. This small amount of differentiation may be more driven by the large distance between these areas (approximately 400 km), compared to shorter geographic distances between sampled areas south of the SLRS. South of the SLRS, genetic differentiation and gene flow results indicated a well-connected, core complex of moose populations, including Maine, New Hampshire, Vermont, and southeastern Quebec. Sampled moose from New York were also connected to this complex (primarily through Vermont, western Maine, and eastern Quebec), also with low differentiation. Despite an increasingly fragmented landscape from urban and agricultural development (Bélanger & Grenier 2002; Thompson et al. 2013; Jeon et al. 2014; Pearman Gillman et al. 2020a,b), this connectivity may have prevented further reductions in genetic diversity across the region.

Gene flow and genetic differentiation were not particularly limited north and south of the SLRS, nonetheless, our results suggest that the wider eastern sections of SLRS act as a barrier to gene flow. There was no evidence that the SLRS blocked connectivity between western Quebec and New York. Krester et al. (2011) found evidence of this barrier in their principal component analysis of moose genotyped with microsatellites across the same region as this study. Our DAPC results supported this conclusion, but our cluster analysis showed a subset of New York moose completely or partially assigned to the predominant western Quebec cluster (and some probability of assignment for moose in southeastern Quebec and northern Maine), and low levels of gene flow across the SLRS between New York and western Quebec. Further east, there was no evidence of connectivity between northeastern Quebec and southeastern Quebec, and northern or western Maine. Northeastern Quebec had the highest degree of genetic differentiation from all other sampled areas, well exceeding F_{st} estimates documented between Alaskan moose populations 900 km apart (Schmidt et al. 2009). Northeastern Quebec also had the lowest levels of gene flow to and from other sampled areas and was dominated by a cluster that was not detected south of the SLRS. Moose have been observed swimming great distances in still water (12–32 km; Peterson 2019; Krefting 1974; Franzmann and Schwartz 2007), but crossing the wide, tidal, and mostly ice-free eastern section of the SLRS may be difficult, potentially explaining the state of the Northeastern Quebec population. Further upstream, narrower western sections of the SLRS would present more likely opportunities for moose to cross the SLRS, such as the sections between the sampled areas in western Quebec and New York.

Moose genetic diversity and connectivity documented in this study may be threatened by future human development across the region (Pearman Gillman et al. 2020a,b) and along the SLRS (Desgranges & Jobin 2003), and climate-mediated shifts in parasite occurrence (Murray et al. 2006; Lankester 2010; Ellingwood et al. 2020; DeBow et al. 2021). The combination of these two pressures could further reduce the limited genetic connectivity across the SLRS, the only link we found between southern and northern *A.a. americana* populations. Human development does not have to be expansive to impact gene flow; Wilson et al. (2015) estimated recent genetic sub-structuring for a moose population that corresponded with a single, heavily used highway. Future human development may pose multiple barriers to wildlife connectivity, including moose. The forecasted patterns of moose occurrence in 2060 under various climate and human development scenarios by Pearman-Gillman et al. (2020a) predicted that moose occurrence in New England may be reduced drastically, with only northern areas of New England maintaining high probabilities of moose occurrence. Increasing barriers to gene flow, combined with potential population reductions due to climate mediated

challenges, may have consequences for future adaptive potential for moose south of the SLRS.

Future research priorities

The current state of genetic diversity and connectivity and forecasted shifts in the distribution of moose raises several important questions for future research. First, how does the observed variation and connectivity in this study contribute to the adaptation and persistence of moose into the future? This is a key question given the context and objectives of this study. With the low diversity observed across the moose' range in North America, our findings may confirm a general pattern for the species rather than indicate a deficit in this subspecies ability to adapt to changing conditions. Further research could prioritize how changes in variation and connectivity have impacted the presence and distribution of unique alleles in moose populations important for adaptation to local conditions (Assis et al. 2013), and broadly examine adaptive capacity across the range of the species (Beever et al. 2016).

Second, if we assume that the observed variation and connectivity plays a role in the persistence of moose in the area, how is genetic connectivity currently being maintained in this region on a scale relevant to habitat management? Connectivity between populations may be jeopardized in discrete area(s) as human development continues across eastern North America. Future research modeling the movement patterns of dispersing individuals across the region and the influences of anthropogenic pressures may be beneficial, as well as research identifying key links between moose populations vulnerable to being severed by projected landscape conditions, particularly links across the SLRS. This information would greatly benefit wildlife habitat management efforts to maintain connectivity between moose populations and slow genetic consequences of population fragmentation.

Third, what are the fitness ramifications for moose given the current state of genetic diversity? Genetic factors likely contribute to how moose cope with the pressures they face along the southern extent of their distribution and could provide some level of resiliency with increasing challenges. For example, there is little knowledge about the relationship between winter tick infestation and pathogenesis and the genetics of individual moose. Aspects of host genetics have been demonstrated in other host-parasite interactions (O'Brien & Evermann 1988; Coltman et al. 1999; Neto et al. 2011; Isomursu et al. 2012; Ruiz-Lopez et al. 2012). These studies range from correlating heterozygosity to reduced ectoparasite loads in wildlife species such as raccoons (*Procyon lotor;* Ruiz-Lopez et al. 2012), the role of inbreeding in parasite communities in Soay sheep (*Ovis aries*; Coltman et al. 1999), to more explicit linkages between ectoparasite burden and chromosomal regions and particular genes in domesticated cattle (*Bos sp.*; Neto et al. 2011). Such information for winter tick and other detrimental parasites could be valuable in the genetic management of moose populations facing increased rates of parasitism. Genes involved with inflammatory response and production of collagen and keratin may aid or hinder the attachment of ticks, as seen in other systems (Neto et al. 2011). There may be genotypes in moose that could influence the severity of winter tick infestation and reduce the physiological burden during tick attachment and feeding. Further, genetic factors contribute to processes that influence population vital rates, including twinning (Van Vleck et al. 1991). If certain populations maintain alleles or diversity metrics associated with increased reproduction rates, this information could greatly benefit conservation efforts across the region.

Finally, how might epigenetic variation contribute to moose being able to persist despite low heterozygosity and high IBS? Heritable epigenetic variation is critical for individuals to cope with environmental pressures, including parasite infestation (Wenzel and Piertney 2014; Mukherjee et al. 2019). Epigenetic variation provides additional plasticity in variable environments and has been linked to genetic differentiation (Fargeot et al. 2021). Moose sampled in this study were not notably differentiated, but future range restrictions and loss of connectivity may influence both genetic and epigenetic variation, compromising how these populations may persist in an increasingly challenging future.

Conclusion

Moose have faced a dynamic history of population colonization and bottlenecks across North America, and currently face unprecedented challenges from climate change, parasite communities, and human development (Hundertmark et al. 2003; Wattles and DeStafeno 2011). Moose have persisted in eastern North America with apparently low genetic diversity and heterozygosity, but these current and future challenges prompt questions about the resiliency of moose populations moving forward. This study presented a comprehensive view of genetic diversity and connectivity across critical moose populations using a novel SNP panel. The rapid advances in our understanding of moose population dynamics, the challenges they face, and the genetic toolsets available provide important information about how current population genetics could be impacted in the future. Further research is needed to better understand the implications of low genetic diversity and potential loss of connectivity for

both demographic and genetic management of moose populations across eastern North America.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10592-022-01496-w.

Acknowledgements This work was supported by Vermont Fish and Wildlife Department in cooperation with the U.S. Fish and Wildlife Service Division of Wildlife and Sportfish Restoration – Wildlife Restoration Program and Safari Club International [06120FY18501]; U.S. Department of Agriculture National Institute of Food and Agriculture McIntire-Stennis Program [1002300]; the University of Vermont's Rubenstein School of the Environment and Natural Resources' Rubenstein Graduate Fellowship; and NRSP8-HATCH Grant VT-H02704MS from the USDA National Institute of Food and Agriculture. This study would not have been possible without the contributions from multiple state and provincial agencies and academic institutions. We are extremely grateful to all the regional biologists and technicians across multiple state and provincial agencies and the faculty and graduate students at multiple universities who sampled all the moose in this study. We thank C. Alexander, N. Fortin, J. DeBow, J. Blouin, P. Pekins, L. Kantar, M. Lavoie, A. Dumont, K. Schuler, D. Wattles., and K. MacKenzie for their collaboration in organizing these samples. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. The Vermont Cooperative Fish and Wildlife Research Unit is jointly supported by the U.S. Geological Survey, University of Vermont, Vermont Fish and Wildlife Department, and Wildlife Management Institute.

Author contributions Elias Rosenblatt, Katherina Gieder, James Murdoch, Therese Donovan, and Stephanie McKay contributed to the study conception and design. Elias Rosenblatt coordinated regional sample collection and submission. Material preparation was performed by Suraj Bhattarai, Emory Pacht, Emma Verbist and Stephanie McKay. Analyses were performed by Elias Rosenblatt, Stephanie McKay, and Brenda M. Murdoch. The first draft of the manuscript was written by Elias Rosenblatt and Stephanie McKay, and all authors commented and contributed to previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by Vermont Fish and Wildlife Department in cooperation with the U.S. Fish and Wildlife Service Division of Wildlife and Sportfish Restoration – Wildlife Restoration Program and Safari Club International [06120FY18501]; U.S. Department of Agriculture National Institute of Food and Agriculture McIntire-Stennis Program [1002300]; and NRSP8-HATCH Grant VT-H02704MS from the USDA National Institute of Food and Agriculture. Author E.R. received support from the University of Vermont's Rubenstein School of the Environment and Natural Resources' Rubenstein Graduate Fellowship.

Data availability The dataset generated and analyzed during the current study are openly available in USGS ScienceBase at https://doi.org/10.5066/P9B30K2L.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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