# Population genetic structure of the provincially endangered mainland Eastern Moose (*Alces americanus americanus*) in Nova Scotia, Canada

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## Abstract
Eastern Moose (*Alces americanus americanus* (Clinton, 1822)) on mainland Nova Scotia (MNS) are declining and experience limited immigration across the Isthmus of Chignecto from the larger population in neighbouring New Brunswick. Provincially Endangered, the recovery strategy for MNS Moose involves mitigating various threats that may lead to local extirpation. We examine genetic diversity of MNS Moose using microsatellite markers and mitochondrial (mtDNA) control region sequences. Genetic similarities with the *Alces a. americana* population in New Brunswick and the introduced Northwestern Moose (*Alces americanus andersoni* (= *Alces alces andersoni*) Peterson, 1952) population on Cape Breton Island are also analysed. Observed heterozygosity for microsatellites for MNS Moose was low and there was also evidence of limited gene flow between Nova Scotia and New Brunswick across the narrow Isthmus of Chignecto that connects these provinces. Consistent with relatively recent colonization of North America by Moose dispersing across the Bering Land Bridge <15 000 years ago, mtDNA haplotypes of MNS Moose were identical or extremely similar to haplotypes found across North America. However, mtDNA diversity was lower in Nova Scotia and New Brunswick than in more central regions of the species' range. Active measures to maintain habitat that promote connectivity across the Isthmus of Chignecto would likely be valuable for Moose in terms of maintaining genetic variation in the region and reducing inbreeding.
Key words: *Alces americanus americanus*; Isthmus of Chignecto; microsatellites; mitochondrial DNA control region; population genetic structure; provincially Endangered Moose

## Introduction

The likelihood of population persistence can be compromised by genetic drift in small and isolated populations that experience decreased genetic diversity and increased inbreeding (Grueber *et al.* 2008; Frankham *et al.* 2010). Although inbreeding depression (ID) may not have sufficient time to affect rapidly declining populations, O'Grady *et al.* (2006) concluded in their meta-analysis that the effect of ID is a major extinction threat to small and moderate-sized populations (less than a few thousand individuals). Decreased genetic diversity and ID is related to reduced population fitness (Reed and Frankham 2003; Poirier *et al.* 2019) with an expectation of low

potential for small populations to adapt to environmental changes (Vander Wal *et al.* 2012; Willi *et al.* 2022), although such outcomes are not necessarily a certainty (Teixeira and Huber 2021).

Deterministic threats (e.g., habitat loss, fragmentation, mortality) and stochastic factors (e.g., demography, genetic and environmental stochasticity, disease) associated with population bottlenecks in small, fragmented, and declining populations can lead to local extirpation of a species (O'Grady *et al.* 2004; Brook *et al.* 2008; Frankham 2015, 2016). Populations should have an effective population size ($N_e$), or be connected by gene flow to subpopulations with a total $N_e$ that exceeds 1000 individuals (Weeks *et al.*

2011). An $N_e = 1000$ is described as the minimum threshold to maintain adaptive potential and evolutionary resilience in a broad array of organisms from plants to insects to mammals (Willi *et al.* 2006). Eastern Moose (*Alces americanus americanus* (Clinton, 1822) = *Alces alces americana*) on the mainland of Nova Scotia, Canada may be experiencing a population bottleneck and potentially changes in their genetic diversity compared to the larger population in neighbouring New Brunswick. The taxonomic name used here follows Bradley *et al.* (2014) citing Boeskorov (2003) that differentiates *Alces americanus* (Moose) as distinct from *Alces alces* (Eurasian Elk).

The Isthmus of Chignecto (Figure 1) links mainland Nova Scotia to continental North America and is the most probable historical migration route into the province for Moose following the deglaciation of the Laurentide Ice Sheet in Atlantic Canada and New England ~12 000 years before present (BP; Shaw *et al.* 2002, 2006). Combined glacio-isostatic, eustatic, and hydro-isostatic processes maximized the width of the isthmus between 10 000–8000 years BP (Shaw *et al.* 2002), the latter period corresponding to the scenario proposed by Hundertmark and Bowyer (2004) for Moose colonization of eastern North America from a centrally located population (Hundertmark *et al.* 2003). Increased tidal amplitudes in the Bay of Fundy (~7000 years BP), opening of the Northumberland Strait (~6000 years BP), and formation of extensive tidal marshes at the head of Chignecto Bay (beginning ~3000 years BP) reduced the isthmus close to its current 21 km width (Shaw *et al.* 2002, 2010). Dyking of the extensive salt marsh for agriculture in the late 1600s, harvesting on adjacent forested uplands, and urban/rural development have significantly altered the habitat of the isthmus such that modelled connectivity corridors for terrestrial-based species between the Nova Scotia border and the rest of continental North America are confined to a narrow, 5 km link (Nussey and Noseworthy 2018).

Before European contact, Moose were the most abundant cervid species in Nova Scotia and New Brunswick (Francis 2018). Native Moose were apparently extirpated from Cape Breton Island (Nova
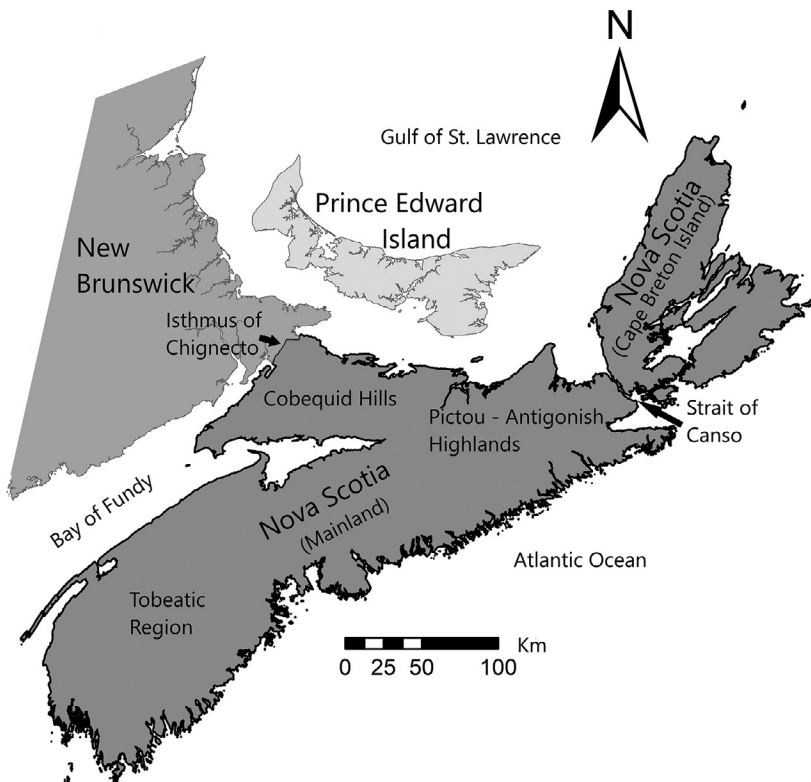


**FIGURE 1.** The Maritime provinces of Canada, showing features identified in the text. The locations of the three localized groups identified in the "Recovery Plan for the Moose (*Alces alces americana*) in Mainland Nova Scotia" (NSDNRR 2021b) are indicated as the Cobequid Hills near the Nova Scotia-New Brunswick border, the Pictou-Antigonish Highlands in northeastern Nova Scotia, and the Tobeatic Region in southwestern Nova Scotia, respectively.

Scotia) in the late 1800s–early 1900s (Corbett 1995; Pulsifer and Nette 1995). The population of Eastern Moose on Mainland Nova Scotia (MNS), is distinct from the introduced subspecies of Northwestern Moose (*Alces alces andersoni* Peterson, 1950) present on Cape Breton Island (CBI). According to Boyer (1950) cited in Bridgland *et al.* (2007), eight Moose were translocated from Elk Island National Park, Alberta, to the east side of Cape Breton Highlands National Park in 1947 and 10 more were introduced to the same location in 1948. The Nova Scotia Department of Natural Resources and Renewables (NSDNRR) estimates that there are currently ~5000 Moose on CBI (NSDNRR 2021a). Moose on MNS have experienced a dramatic decline and the current population is likely less than the minimum sustainable $N_e$ threshold described by Willi *et al.* (2006). Parker (2003) estimates the pre-European contact population of MNS Moose at ~15 000 animals. A significant decline in numbers by the mid 1970s (Parker 2003 and references therein) continued through the 1990s. Although estimated to be ~1000–1200 animals when listed as provincially Endangered in 2003 under the *Nova Scotia Endangered Species Act*, Brannen (2004) estimated the mainland population at fewer than 700 individuals distributed primarily among three localized groups (a group in the Cobequid Hills of Cobequid and Cumberland counties near the border of Nova Scotia and New Brunswick, a northeastern group in the Pictou-Antigonish Highlands, and a southwestern group in the Tobeatic Region; see Figure 1; Pulsifer and Nette 1995; Parker 2003; Snaith and Beazley 2004). Surveys and coverage have been insufficient, coupled with very low animal densities, to provide a statistically valid estimate of the current population among the three areas isolated by anthropogenic habitat alterations, major highways, and urban and agricultural development (NSDNRR 2021b).

The MNS Moose is increasingly isolated from the larger population in New Brunswick that could be used for genetic rescue or genetic restoration (*sensu* Weeks *et al.* 2011) as a conservation strategy where the risk of outbreeding depression is low (Ralls *et al.* 2018). The harvestable population on CBI is separated from the MNS Moose population by the narrow Strait of Canso which may not be a barrier to the two populations interbreeding as Moose have been reported swimming between parts of CBI and MNS (Bridgland *et al.* 2007). We examine the genetic diversity in nuclear and mitochondrial (mt) DNA within NSM Moose from the Cobequid Hills and Pictou-Antigonish Highlands, and we assess similarity with the *A. a. americanus* population in New Brunswick and the introduced *A. a. andersoni* population on Cape Breton Island.

## Methods

### Sample collection and processing

We obtained all tissue samples from necropsied Moose specimens collected by provincial government agencies and so, in accordance with Category of Invasiveness A of the Canadian Council on Animal Care, no separate animal care protocol was required for the genetic analyses we conducted. For our study, no samples were available from the Tobeatic Region localized group; all samples of MNS Moose were from either the Cobequid Hills or the Pictou-Antigonish Highlands localized groups. As will be noted in our Results, we found no evidence of significant genetic differentiation between Moose from the Cobequid Hills and Pictou-Antigonish Highlands so these localized groups are simply referred to as northeast MNS Moose hereafter in this analysis. We collected samples for CBI from ear tissue of 87 Moose harvested in 2018 and skeletal muscle tissue from nine necropsied Moose stored at −20°C at the Atlantic Veterinary College (AVC). We obtained tongue samples from 32 Moose harvested in 2018 in southern New Brunswick (NB). Skeletal muscle tissue from 66 necropsied Moose stored at −20°C at the AVC provided the samples for northeast MNS Moose.

We processed ear tissue as follows. Ears arrived whole from which we removed hair using a sterile razor blade. A 3×3×3 mm triangle was cut and a layer of skin was removed and used as tissue for DNA extraction. Skeletal muscle tissue collected from the quadriceps muscle were delivered from the AVC as 5×5×5 mm cubes of muscle tissue stored in ethanol. Small interior sections of tissue were used for the DNA extraction. We obtained tongue samples by dissecting through the tongue mucosa and submucosa and collecting portions of the underlying skeletal muscle. We stored scalpel blades and razors in 10% bleach solution between uses. Prior to use we immersed them in 100% ethanol, rinsed with distilled water, and held over a flame to prevent sample cross contamination.

We used a DNeasy Blood and Tissue kit (Qiagen, Germantown, Maryland, USA) to perform DNA extractions following manufacturer's protocol. We conducted polymerase chain reactions (PCRs) on 10 microsatellite loci amplified in two multiplexes as described by Ball *et al.* (2011): Multiplex 1: MAP2C, RT9, RT24, BM1225, BM4513 and Multiplex 2: RT30, FCB193, BM888, BM848, BL42. The 10 µL reaction mix for Multiplex 1 contained 3 µL of DNA template, 0.5 U of taq and 1× concentration of PCR buffer (Invitrogen, Frederick, Maryland, USA), 0.2 mM of dNTPs, 1.5 mM of $MgCl_2$, 0.2 µg of BSA, with primers and fluorescent dyes at the following concentrations: 500 nM of MAP2C primers with Fam

label, 500 of RT9 (Hex), 400 nM of RT24 (Hex), 200 nM of BM1225 (Fam), and 200 nM of BM4513 (Fam). The 15 µL reaction mix for Multiplex 2 contained 3 µL of DNA template, 1× concentration of Multiplex Mastermix (Qiagen) with primers and fluorescent dyes at the following concentrations: 400 nM of RT30 with NED label, 300 nM of FCB193 (NED), 300 nM of BM888 (Fam), 500 nM of BM848 (Fam), and 500 nM of BL42 (Ned). Cycling conditions were 95°C for 15 min, followed by 30 cycles of 94°C for 30 s, 60°C for 90 s, 72°C for 60 s with a final extension at 60°C for 30 min.

We amplified the hypervariable domain of the mtDNA control region with primers LGL283 and ISM015 (Hundertmark *et al.* 2002). The 20 µL reaction mix contained 2 µL of DNA template, 1 U of taq (Invitrogen), 1.5 mM MgCl$_2$, 0.2 mM dNTPs, 200 nM of primers, and 0.2 µg of BSA. Cycling conditions were 94°C for 5 min, 30 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 30 s with a final extension of 72°C for 2 min. We visualized amplified products on a 1.5% agarose gel and purified with ExoSAP; the Sanger sequencing reactions were performed at the McGill University and Génome Québec Innovation Centre.

Polymerase chain reactions and genotyping were completed at Trent University in the Natural Resources Wildlife DNA Profiling and Forensic Centre. All microsatellite amplifications were analyzed on an ABI 3730 genetic analyzer (Applied Biosystems, Foster City, California, USA) using Genescan ROX500 size standard (Applied Biosystems).

*Microsatellite data analyses*

A list of all programs that we used in the microsatellite data analyses is provided in Table 1. Allele peaks were scored with Genemarker v1.95. We used Cervus to estimate frequency of null alleles in each locus and to estimate the probability of identity using both the probability a genotype at a locus is identical between unrelated individuals (PID) as well as between full siblings (PIDsib). We also used Cervus

to estimate regional mean observed and expected heterozygosity for: 1) CBI, 2) MNS, 3) NB, and 4) Moose from all regions combined, and to calculate the Polymorphic Information Content value (PIC) for each microsatellite locus, where PIC is a measure of the utility of a polymorphic molecular marker to infer relatedness and other population genetic parameters. We used the program *FST*AT to estimate allelic diversity ($N_A$), allelic richness ($A_r$), which corrects for sample size to facilitate comparisons across different studies (Goudet 2003), and inbreeding coefficient ($F_{IS}$) for each regional grouping.

We initially investigated population structure using the Hardy-Weinberg Exact Test and default settings in the package "Genepop" written in R (R Core Team 2023). We did this for: 1) each region/localized group separately, 2) MNS and NB Moose combined, and 3) for the entire data set. We used *FST*AT to estimate genetic differentiation between each region using $F_{ST}$ values. Two separate runs applying the same settings in STRUCTURE were used to assess population structure in the data set for 1) all regions, and 2) NB and MNS Moose. Admixture was assumed with a Burn-in Period Length of 100 000 and 100 000 Markov chain Monte Carlo (MCMC) repetitions using the Allele Frequencies Correlated Model. We set the number of potential populations ($K$) to 1–10 with 10 iterations of each value. The most likely number for populations was evaluated using both the Evanno Method (Delta $K$) through the Structure Harvester web interface as well as assessing at which value of $K$ that Posterior probability [Ln P(D)] begins to stabilize. To examine for evidence of potential barriers to population movement across the Isthmus of Chignecto, the presence of the isolation by distance (IBD) pattern was first assessed across the geographical scale of NB to MNS Moose. We also completed an IBD assessment between the Cobequid Hills and Pictou-Antigonish Highlands localized groups of MNS Moose. For samples from southern New Brunswick, we used the centroids of the management zones

**TABLE 1.** Programs used in microsatellite and mitochondrial DNA analyses.

| Program | Website |
|---|---|
| Genemarker | https://genemarker.software.informer.com/1.9/ |
| Cervus | http://www.fieldgenetics.com |
| FSTAT | https://www2.unil.ch/popgen/softwares/fstat.htm |
| Genepop | https://cran.r-project.org/web/packages/genepop/index.html |
| STRUCTURE 2.3.4 | https://web.stanford.edu/group/pritchardlab/structure.html |
| GenAlx 6.5 | https://biology-assets.anu.edu.au/GenAlEx/Download.html |
| DNAsp | http://www.ub.edu/dnasp// |
| Arlequin | http://cmpg.unibe.ch/software/arlequin35/ |
| MEGAX | https://www.megasoftware.net/ |

from which the animals were harvested as their location when this was the only available geographic information. For the Nova Scotia samples, their precise geographic location information was available. We conducted these assessments in GenAIx 6.5 using a Mantel test with 99 permutations.

*Mitochondrial DNA data analyses*

The list of programs we used in the mtDNA data analyses is provided in Table 1. We used MEGAX to visualize electropherograms, to manually assess the quality of each sequence, and to trim and align sequences. We used DNAsp and Arlequin to identify polymorphic sites in the dataset and to assess genetic diversity for all regions as well as each region individually. Measures included the number of haplotypes (h), haplotype distribution, haplotype diversity ($H_d$), nucleotide diversity ($P_i$), and average number of nucleotide differences between individuals (k). We used DNAsp to compare the number of nucleotide differences between regions ($K_{xy}$) and estimate genetic differentiation between populations ($G_{ST}$). We also used DNAsp to perform a $\chi^2$ permutation test with 1000 replicates to test for genetic differentiation between population pairs.

## Results

*Microsatellite data–genetic diversity estimates and population structure*

In total, 55, 32, and 96 samples were used for microsatellite genotype analyses from MNS, NB, and CBI Moose, respectively. Although the number of samples varied, all regions had samples of over 30. According to Hale *et al.* (2012), 25–30 individuals is sufficient for accurate analysis of population structure using microsatellites. The average frequency of null alleles was 0.103, with the highest being 0.3113 for locus MAP2C (Table 2). PIC ranged from 0.260 to 0.805, with an average of 0.575 (Table 2).

CBI Moose samples had slightly higher observed heterozygosity, $H_o$ (0.547 ± 0.123), than either MNS (0.488 ± 0.163) or NB (0.460 ± 0.119) Moose (Table 3). All regions had a similar average number of alleles per locus (range ~4–4.5 alleles/locus) and similar allelic richness (range ~3.8–4.3 alleles/locus). All regions had $F_{IS}$ values close to zero, with the NB Moose samples having the highest $F_{IS}$, and MNS Moose samples having a slightly negative value. Genotypes in the sample set had low probability of being identical (PID = $2.000 \times 10^{-8}$; PIDsib = $6.832 \times 10^{-4}$).

The $F_{ST}$ was lowest when comparing MNS to NB Moose samples (0.0716). The $F_{ST}$ comparisons were considerably higher when comparing either MNS to CBI (0.2877) or NB to CBI (0.2473) Moose. Departures from Hardy-Weinberg Equilibrium (HWE) were not significant (i.e., $P > 0.05$) for samples within their respective regions of MNS ($P = 0.160$), CBI ($P = 0.089$), and NB ($P = 0.053$) Moose. There were, however, significant departures from HWE expectations for combined MNS and NB ($P = 0.038$) and for MNS, NB, and CBI Moose combined ($P = 0.015$). Given that we detected null alleles, and that null alleles for microsatellites may lead to departures from HWE (Brookfield 1996), null alleles could be a factor in our analyses. However, because there were not statistically significant departures from HWE within regions but only between regions, we interpret this result as evidence of true genetic differentiation between regions. When STRUCTURE was run with all regions combined, nLnaP(D)f began leveling off

**TABLE 2.** Estimates of null allele frequencies and Polymorphic Information Content (PIC) of microsatellite loci estimated using Cervus.

| Locus | Est. freq. null alleles (Cervus) | PIC |
|---|---|---|
| MAP2C | 0.3113 | 0.504 |
| BM4513 | 0.1296 | 0.703 |
| BM1225 | 0.0567 | 0.626 |
| RT9 | 0.1175 | 0.784 |
| RT24 | 0.0610 | 0.501 |
| BM888 | 0.0143 | 0.260 |
| BM848 | 0.1466 | 0.572 |
| FCB193 | 0.0109 | 0.634 |
| RT30 | 0.1653 | 0.359 |
| BL42 | 0.0210 | 0.805 |

**TABLE 3.** Microsatellite genetic diversity measures ($H_o$ = observed heterozygosity, $H_e$ = expected heterozygosity, $F_{IS}$ = Inbreeding Coefficient) for Moose (*Alces alces*) in mainland Nova Scotia (MNS), Cape Breton Island (CBI), and New Brunswick (NB).

| Region | Mean $H_o$ (Cervus) | Mean $H_e$ (Cervus) | Avg. # of Alleles/ locus (FSTAT) | Allelic richness (FSTAT) | $F_{IS}$ (FSTAT) |
|---|---|---|---|---|---|
| CBI | 0.547 ± 0.123 | 0.550 ± 0.115 | 4.4 ± 0.837 | 3.825 ± 0.715 | 0.007 |
| MNS | 0.488 ± 0.163 | 0.476 ± 0.159 | 4.0 ± 1.700 | 3.816 ± 0.963 | −0.024 |
| NB | 0.460 ± 0.119 | 0.500 ± 0.144 | 4.4 ± 1.313 | 4.380 ± 1.305 | 0.081 |
| Overall | 0.510 ± 0.110 | 0.619 ± 0.109 | 6.0 ± 1.307 | 5.144 ± 1.068 | |

at $K = 2$ (Figure 2a). A similar result was found using the Evanno Method, with Delta $K$ being the largest (> 1500 on the y-axis indicating a strong level of population differentiation) at $K = 2$ (Figure 2b). The Q plot derived from STRUCTURE when $K = 2$ divided individuals into two groups: 1) samples from CBI, and 2) MNS and NB (Figure 2c). The pattern of Ln P(D) over values of $K$ when only samples from MNS and NB were included indicated $K = 1$ (Figure 3a). Although Delta $K$ was highest at $K = 2$, the Delta $K$ value was low at 25, consistent with limited differentiation (Figure 3b). In the Q plot for NB and MNS Moose alone with $K = 2$, NB samples were predominately grouped together and MNS samples were predominately grouped together, with some individuals from each region showing genetic signatures from the opposite region (Figure 2d). Evidence of a weak but significant presence of IBD ($r^2 = 0.019$, $P = 0.03$) occurred between all samples from NB and MNS

but not between samples from the Cobequid Hills and Pictou-Antigonish Highlands localized groups of MNS Moose, respectively ($r^2 = 0.002$, $P = 0.23$).

*Mitochondrial DNA data*

After assessing quality, 50, 30, and 85 mtDNA sequences were usable from MNS, NB, and CBI Moose, respectively. After sequences were trimmed of primers and uncertain nucleotides adjacent to the primers, they were 479 nucleotides long. The sample set from MNS tended to show somewhat higher levels of haplotype diversity when compared to the other regions within our study (Table 4). Six haplotypes (referred to here as Hap 1 to Hap 6) were found in this region, with the haplotypes in CBI being distinct from those found in the other two regions (Table 5). Each of these six haplotypes was compared to sequences available in the GenBank database using NCBI Blast (blast.ncbi.nlm.nih.gov) for comparison with haplotypes identified in Hundertmark *et al.* (2003). Hap 1
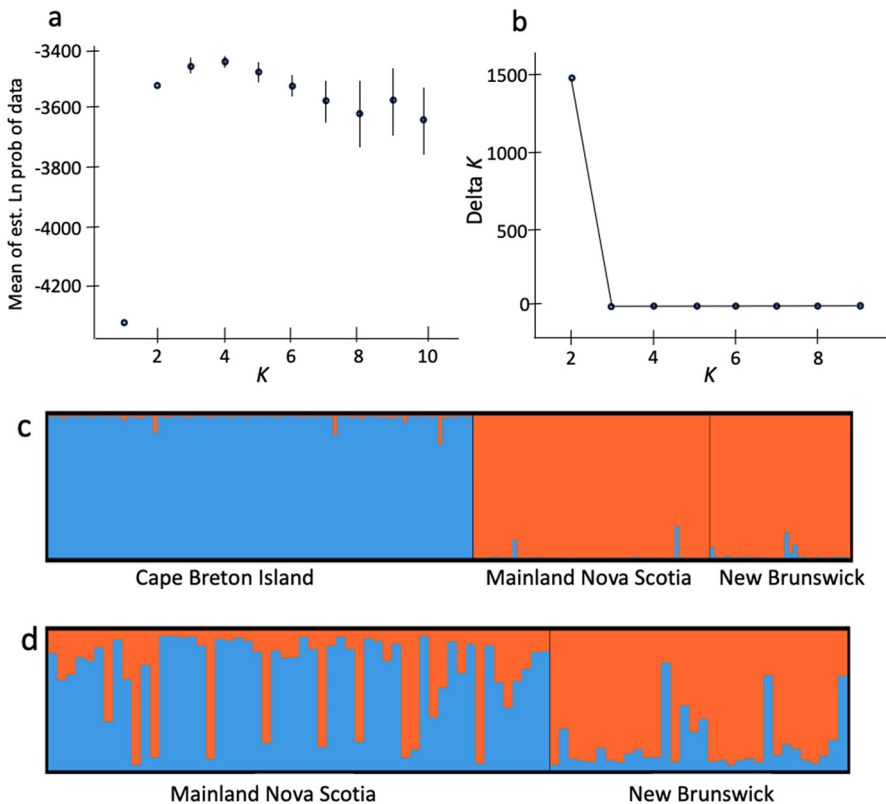


**FIGURE 2.** a. Change in mean Posterior probability [LnP(D)] in Moose (*Alces alces*) as a function of the number of subpopulations $K$ over values 1–10 for all regions together (Cape Breton Island [CBI] + Mainland Nova Scotia [MNS] + New Brunswick [NB]), with SD bars calculated by STRUCTURE. b. Evanno Method (Delta $K$) results when all regions (CBI+MNS+NB) are combined in STRUCTURE. c. Q plot for STRUCTURE run with all regions combined when $K = 2$. d. Q plot for STRUCTURE run with genotypes from mainland Nova Scotia (MNS) and New Brunswick (NB) combined when $K = 2$.
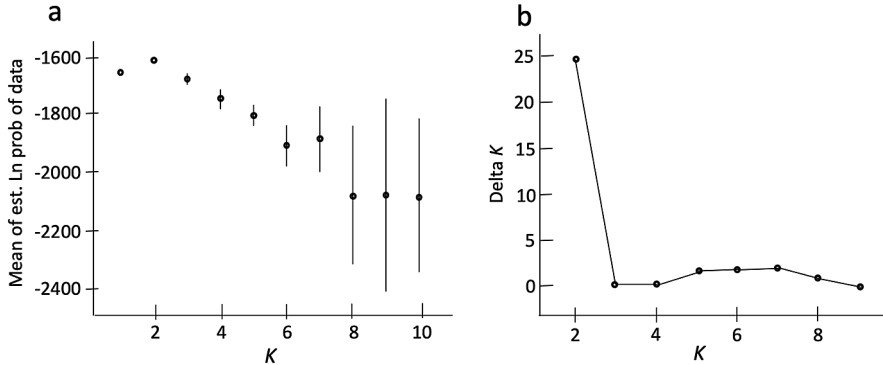
**FIGURE 3.** a. Change in mean Posterior probability [LnP(D)] in Moose (*Alces alces*) as a function of the number of subpopulations $K$ over values 1–10 when samples from mainland Nova Scotia (MNS) and New Brunswick (NB) were analyzed, with SD bars calculated by STRUCTURE. b. Evanno Method (Delta $K$) results for New Brunswick (NB) and mainland Nova Scotia (MNS) in STRUCTURE.

**TABLE 4.** Mitochondrial DNA genetic diversity measure outputs from DNAsp (h = number of haplotypes, $H_d$ = haplotype diversity, $P_i$ = nucleotide diversity, k = average number of nucleotide differences within a population, '±' is SD) for Moose (*Alces alces*) in mainland Nova Scotia (MNS), Cape Breton Island (CBI), and New Brunswick (NB).

| Region | h | $H_d$ | $P_i$ | k |
|--------|---|-------|-------|---|
| CBI | 2 | $0.430 \pm 0.039$ | $0.90 \times 10^{-3} \pm 0.8 \times 10^{-4}$ | 0.430 |
| MNS | 4 | $0.520 \pm 0.044$ | $2.17 \times 10^{-3} \pm 1.9 \times 10^{-4}$ | 1.042 |
| NB | 3 | $0.441 \pm 0.098$ | $1.50 \times 10^{-3} \pm 0.4 \times 10^{-3}$ | 0.717 |
| Overall | 6 | $0.750 \pm 0.014$ | $2.65 \times 10^{-3} \pm 0.9 \times 10^{-4}$ | 1.269 |

**TABLE 5.** Haplotype distribution of Moose (*Alces alces*) samples from Cape Breton Island (CBI), mainland Nova Scotia (MNS; including the Cobequid Hills and Pictou-Antigonish Highlands localized groups), and southern New Brunswick (NB).

| Haplotype | CBI | MNS | NB |
|-----------|-----|-----|-----|
| Hap 1 | 0.694 | 0.000 | 0.000 |
| Hap 2 | 0.306 | 0.000 | 0.000 |
| Hap 3 | 0.000 | 0.600 | 0.135 |
| Hap 4 | 0.000 | 0.020 | 0.135 |
| Hap 5 | 0.000 | 0.360 | 0.733 |
| Hap 6 | 0.000 | 0.020 | 0.000 |

and Hap 2 were identical or nearly identical (differing by only a single nucleotide) to the haplotype referred to as "Alaska1" (accession no. AF412235.1) on GenBank that references Hundertmark *et al.* (2002). Hap 1 and Hap 2 are also identical or nearly identical to a series of haplotypes from a study by DeCesare *et al.* (2020) from various states, provinces, and territories in western North America. Hap 3 was identical to the haplotypes referred to as "Central3" (AF412242.1) and "Central4" (AF412244.1) on GenBank. In Hundertmark *et al.* (2003), "Central" samples were from northeastern and northcentral Minnesota, southwestern Ontario, Isle Royale, Michigan, northeastern North Dakota, and the Lake Winnipeg area of Manitoba, which cover the range of subspecies *A. a. andersoni*. Hap 4 and Hap 5 were identical to haplotypes "East2" (AF412243.1) and "East1" (AF412239.1), respectively. Lastly, Hap 6 was nearly identical to haplotypes Central3 and Central4. The "Eastern" haplotypes of Hundertmark *et al.* (2003) were from New Hampshire and New Brunswick and represent *A. a. americana*. Note: the reason that a haplotype from the present study, e.g., Hap 6, could be identical to two different haplotypes on GenBank is because the length of the haplotype sequences presented here is slightly shorter than the haplotype length in Hundertmark *et al.* (2003). NB and CBI Moose had the largest average difference in nucleotides (Table 6). Chi-square results supported significant differentiation in haplotype frequencies for all pairwise regional comparisons (CBI–MNS: $\chi_5^2 = 135$, $P < 0.05$; CBI–NB: $\chi_4^2 = 115$, $P < 0.05$; MNS–NB: $\chi_3^2 = 19.288$, $P < 0.05$).

## Discussion

Consistent with values obtained for microsatellites obtained by Ball and Wilson (2003) that are summarized in Table 7, the Moose on CBI had the highest

**TABLE 6.** Nei's measure of genetic diversity ($G_{ST}$; Hudson et al. 1992) and DNAsp output for average number of differences between nucleotides among Moose (*Alces alces*) individuals ($K_{xy}$) of mainland Nova Scotia (MNS), Cape Breton Island (CBI), and New Brunswick (NB).

| Region | $K_{xy}$ | $G_{ST}$ |
|---|---|---|
| CBI-MNS | 1.734 | 0.346 |
| CBI-NB | 1.827 | 0.336 |
| NB-MNS | 1.344 | 0.142 |

level of observed heterozygosity in our analysis, followed by MNS and then NB. The observed heterozygosity for microsatellites for MNS Moose was lower than values typically observed in populations from larger geographic regions such as northwestern Ontario, Manitoba, Finland, and Poland (Table 7). Comparatively, the lower heterozygosity level for MNS Moose is more typical of island or peninsular populations such as Isle Royale, Michigan (Sattler *et al.* 2017) and the Kenai Peninsula, Alaska (Wilson *et al.* 2015; see Table 7), which likely experience restricted gene flow as was noted by the authors of these studies. Similar to both the Isle Royale and Kenai Peninsula populations, mainland Nova Scotia is geologically nearly an island, but it is technically a peninsula as it is connected to New Brunswick by the narrow Isthmus of Chignecto (MacDonald and Clowater 2005). Samples from Nova Scotia and

New Brunswick had similar, relatively low numbers of alleles (between ~4.0 and 4.4), which are considerably lower than the value observed in Ontario and Manitoba that ranged between 6.3 and 9.5 (Table 7). Genetic diversity levels in our study are higher than that observed for insular Newfoundland (Broders *et al.* 1999).

The Newfoundland Moose population was introduced from extremely small numbers of individuals, specifically, one male and one female from Nova Scotia in 1878, and two males and two females from New Brunswick in 1904. Not surprisingly, this founder event resulted in a considerable loss of genetic variation in that population (Broders *et al.* 1999). While the genetic diversity of Newfoundland Moose continues to be low, its population is estimated to be 110 000 individuals and remains stable with only a 4% decline over 10 years between 2012 and 2022 (NLDFFA 2022). While there may be similarities in several of the factors affecting mortality and health of Newfoundland and MNS Moose, unlike Newfoundland Moose that are not exposed to White-tailed Deer (*Odocoileus virginianus*), MNS Moose and White-tailed Deer are sympatric species. This is significant because White-tailed Deer in Nova Scotia are the natural host of the endoparasite Brainworm (*Parelaphostrongylus tenuis*) that is well known to cause significant mortality in Nova Scotia Moose populations, but does not harm White-tailed Deer (Benson

**TABLE 7.** Studies reporting estimates of genetic diversity measures in Moose (*Alces alces*) populations.

| Region | Reference | Mean $H_o$ | Mean $H_e$ | $N_A$ | $F_{IS}$ |
|---|---|---|---|---|---|
| Cape Breton Island, NS | Ball and Wilson 2003 | 0.6073 | 0.6008 | 4.5 | −0.110 |
| Guysborough County, NS | Ball and Wilson 2003 | 0.4405 | 0.5888 | 3.3 | 0.267 |
| Cumberland County, NS | Ball and Wilson 2003 | 0.4265 | 0.4833 | 4.1 | 0.121 |
| Tobeatic, NS | Ball and Wilson 2003 | 0.3889 | 0.4908 | 3.6 | 0.218 |
| New Brunswick | Ball and Wilson 2003 | 0.4500 | 0.5246 | 3.8 | 0.146 |
| Cape Breton Island, NS | Ball *et al.* 2011 | 0.6136 | 0.5968 | 4.5 | |
| Mainland, NS | Ball *et al.* 2011 | 0.4446 | 0.5207 | 4.4 | |
| New Brunswick | Ball *et al.* 2011 | 0.4793 | 0.5355 | 3.7 | |
| Newfoundland | Wilson *et al.* 2003 | | 0.3780 | 2.2 | 0.017 |
| Northwestern Ontario | Price 2016 | 0.5860 | 0.6512 | 8.8 | 0.112 |
| Northeastern Ontario | Price 2016 | 0.5367 | 0.5751 | 9.5 | 0.062 |
| Southcentral Ontario | Price 2016 | 0.4810 | 0.5131 | 7.2 | 0.063 |
| Manitoba | Price 2016 | 0.7014 | 0.6982 | 6.3 | 0.005 |
| Northern Yellowstone, USA | Koitzsch *et al.* 2014 | 0.4885 | 0.4979 | 3.9 | |
| Isle Royale, Michigan, USA | Sattler *et al.* 2017 | 0.4700 | 0.4800 | 4.1 | 0.160 |
| Anchorage, Alaska, USA | Wilson *et al.* 2015 | 0.5200 | 0.5200 | 4.1 | 0.010 |
| Kenai Peninsula, Alaska, USA | Wilson *et al.* 2015 | 0.4200 | 0.4300 | 3.2 | 0.031 |
| Finland, Europe | Kangas *et al.* 2013 | | 0.7400 | 4.7 | −0.004 |
| Poland, Europe | Świslocka *et al.* 2015 | 0.7690 | 0.7810 | 6.4$_r$ | 0.112 |

$X_r$ = Allelic Richness rather than Avg. # of Alleles/Locus.

1958a,b; Smith *et al.* 1964; Smith and Archibald 1967; Thomas and Dodds 1988; Beazley *et al.* 2006). Additionally, White-tailed Deer in Nova Scotia are the natural host of the ectoparasite Winter Tick (*Dermacentor albipictus*), and while White-tailed Deer are unaffected by Winter Tick, this parasite is also reported to be a significant mortality factor in northeastern North American Moose populations (Jones *et al.* 2019). Winter Ticks have been reported on MNS Moose and Winter Tick infestations have been found as a cause of MNS Moose mortality (Beazley *et al.* 2006; Canadian Wildlife Health Cooperative unpubl. data, accessed 20 May 2023).

The viability of individuals and populations is not affected by the loss of genetic variation alone, but through its interactions with demographic and ecological processes (Lacy 1997). The decline of MNS Moose is believed to be multifactorial, likely the consequence of several direct and indirect factors (Beazley *et al.* 2006; NSDNRR 2021b). Therefore, while Newfoundland and MNS Moose both have low genetic diversity, there are other significant factors that lack commonality. Morbidity and mortality of MNS Moose associated with these parasites of sympatric White-tailed Deer are examples of potential cumulative effects that, in conjunction with low genetic diversity, could be facilitating or causing the continued decline of the MNS Moose population. In comparison, freedom of Newfoundland Moose from these parasites could potentially prevent or reduce the consequences of decreased genetic variation in their population.

The $F_{IS}$ values indicated potential inbreeding in the NB Moose population. This result is somewhat surprising given that Moose appear to be abundant in New Brunswick numbering around 29 000 (Nature Conservancy of Canada 2023) and because of the long land borders between New Brunswick, Maine, and Quebec, dispersal of Moose, and consequently gene flow, should be much greater than in Nova Scotia. However, the samples incuded in our analysis were from the southern region of New Brunswick, which is the most densely populated part of the province. Analyses of New Brunswick Moose from central and northern regions of the province may present a different pattern.

Moose samples from both CBI and MNS had $F_{IS}$ values near zero, indicating little evidence of inbreeding. Values of $F_{ST}$ can range from zero to one, but values for natural mammal populations typically range from near zero to ~0.25 (Storz 1999). Unsurprisingly, the CBI population with $F_{ST}$ values around 0.25 showed little evidence of gene flow with either of the MNS or NB subpopulations. An $F_{ST}$ value of ~0.07 indicated a moderate level of genetic differentiation

between the NB and MNS subpopulations, suggesting only limited gene flow between these locations. Ball and Wilson (2003) examined patterns of gene differentiation among three regions of Nova Scotia: Cumberland County (adjacent to New Brunswick and comparable to the Cobequid Hills localized group referred to in the Recovery Plan for Nova Scotia's Endangered mainland Moose), Guysborough County (comparable to the Pictou-Antigonish Highlands group in northeastern MNS), and the Tobeatic Wilderness Area (the central, southwestern region of MNS near Kejimkujik National Park; NSDNRR 2021b). Ball and Wilson (2003) found the Tobeatic and Guysborough regions to be highly similar, but identified moderate to high levels of differentiation between Cumberland and Guysborough, and Cumberland and Tobeatic regions, respectively. Based on their findings, Ball and Wilson (2003) also suggested that levels of gene flow in the region generally were low to very low, although levels of differentiation were lower between New Brunswick and either Cumberland or Guysborough Counties compared to New Brunswick versus the Tobeatic Region.

When CBI, MNS, and NB Moose were considered together, STRUCTURE analysis indicates two genetic populations, with CBI grouping separately and NB and MNS grouping together (Figure 1c). There is additional support for weak population structure between MNS and NB when CBI is removed, and these two regions are analyzed together in STRUCTURE (Figure 2d). Although STRUCTURE results did suggest that MNS and NB Moose are genetically slightly distinct, the low degree of distinctiveness could be due, in part, to the uneven sample samples for these two regions (i.e., 55 and 32 for MNS and NB, respectively). According to simulation studies performed by Puechmaille (2016), uneven sample sizes can cause STRUCTURE to tend to merge subpopulations that are actually distinct. Future studies of population genetic structure of Moose in Atlantic Canada should aim to use similar sample sizes for all regions where possible.

Significant deviations in HWE occurred when all populations were combined and when the MNS and NB populations were compared to one another. The IBD assessment indicated a significant but weak pattern of IBD between NB and MNS suggesting some restriction to animal movement and thus limited gene flow across the Isthmus of Chignecto. Within MNS, however, IBD results indicate there are no barriers to dispersal between the Cobequid Hills and Pictou-Antigonish Highlands localized groups, but the pattern may simply be the result of the geographic scale being too small to detect IBD in such a large-bodied mammal.

In terms of general trends for mtDNA diversity in Moose, Hundertmark *et al.* (2002, 2003) found

that haplotypes were similar across North America, which was indicative of recent colonization of the continent across the Bering land bridge <15 000 years ago. Colonization of the continent likely consisted of episodes of small numbers of dispersers moving into unoccupied regions with eastern North America being colonized from the central region of the continent more recently than 8000 years ago following the retreat of several proglacial lakes that would have impeded expansion of Moose populations eastward. Despite low overall diversity, populations in central North America tend to have higher diversity than Moose populations closer to the east and west coasts. Our results are consistent with this finding, with lower measures of mtDNA diversity in CBI, MNS, and NB than was found by Hundertmark *et al.* (2003) for Moose populations located nearer to the centre of the species range. However, the Hundertmark *et al.* (2003) study only included 13 Moose from eastern North America, including samples from only New Brunswick and New Hampshire. Haplotype and nucleotide diversity measures for the eastern North American samples in that study were lower than our estimates, likely due to the extremely small sample sizes in Hundertmark *et al.* (2003). Both our results and those of Hundermark *et al.* (2003) indicated that haplotypes from eastern North America (representing subspecies *A. a. americana*) were identical or extremely similar to haplotypes from other regions (and subspecies) such as *A. a. andersoni* from central Canada and North Dakota, Minnesota, and northern Michigan, and Alaskan Moose (*Alces alces gigas* Miller, 1899) from Alaska. As expected, our finding that the CBI Moose population has a different set of haplotypes than those found in MNS and NB is consistent with these Moose being descendants of individuals introduced from Alberta. That there are no shared haplotypes between the CBI and MNS samples is consistent with no genetic exchange occurring between these two Moose populations. Although we found a total of four haplotypes in NB and MNS, they were all nearly identical in sequence, and NB and MNS had the lowest number of nucleotide differences between their populations resulting in low values for $K_{xy}$ and $G_{ST}$. Despite the near identity in DNA sequence of these haplotypes, the statistically significant $\chi^2$ differences in haplotype frequencies among Moose in these three geographic locations is an indication that it is not a single homogeneous population for mtDNA. Although MNS and NB Moose shared three of four haplotypes found in the region, the statistically significant $\chi^2$ test indicated that haplotype frequencies are not homogenous across mainland Nova Scotia and New Brunswick, and female-mediated gene flow (for mtDNA) is limited between these two Maritime provinces. Again, because mtDNA is only transmitted by females, this phylogeographic pattern is consistent with female natal philopatry as documented in Moose and cervids generally (e.g., Colson *et al.* 2016).

*Management implications*

An objective of the Recovery Plan for Nova Scotia's Endangered mainland Moose is to "enhance connectivity to improve genetic health and demographic parameters…" by improving connectivity with the larger subpopulation in New Brunswick and among local breeding individuals (NSDNRR 2021b: 34). Action to improve connectivity through landscape management may, in time, reduce the weak IBD between NB and MNS. However, our results indicate the Moose localized groups in NB and MNS exhibit moderate genetic differentiation with limited gene flow between subpopulations. Further, the two subpopulations have heterogenous mtDNA suggesting limited genetic exchange since their founding. This pattern is partially consistent with phylogeographic patterns documented for Maritime Shrew (*Sorex maritimensis*; Dawe *et al.* 2009). Maritime Shrew is a Canadian endemic species that only occurs in Nova Scotia and New Brunswick (Stewart *et al.* 2002). Both nuclear and mitochondrial markers are consistent with the hypothesis that Maritime Shrew diverged into two subpopulations in Nova Scotia and New Brunswick, respectively, as a consequence of post-Wisconsin glacial ebbs and flows (Dawe *et al.* 2009). Although obviously a much smaller animal with much less dispersal ability than is the case for Moose, Maritime Shrew is adapted to the coastal wetland habitats that connect Nova Scotia and New Brunswick, and populations on either side of the Isthmus of Chignecto appear to be genetically connected. Active measures to maintain appropriate habitat that ensures connectivity across the Isthmus of Chignecto would likely be valuable for Moose, as well as for other species such as Maritime Shrew. While some recent studies have questioned the value of maintaining genetic diversity and reducing inbreeding in management plans for species conservation (e.g., Teixeira and Huber 2021), other studies argue that there is "overwhelming evidence that inbreeding depression is often substantial in natural populations" and that "[s]mall populations suffer from reduced mean performance due to the accumulation of deleterious mutations" (Willi *et al.* 2022: 4).

Augmenting gene flow through translocations is a conservation method recommended for threatened species programs to alleviate detrimental genetic effects that arise in small, fragmented populations (Weeks *et al.* 2011; IUCN 2013; Ralls *et al.* 2018; García-Dorado and Caballero 2021). This maintains

genetic diversity at levels similar to large locally adapted populations (Weeks *et al.* 2015). Although Frankham (2015) reports the out-crossing of inbred populations in his meta-analysis resulted in beneficial effects, the translocation of individuals from genetically distinct populations and the concerns over outbreeding depression (Frankham *et al.* 2011) remains controversial due to cultural, taxonomic, and legislative barriers (Love Stowell *et al.* 2017). Ralls *et al.* (2018) argues the hesitancy in outcrossing/gene pool mixing (Weeks *et al.* 2015) by agencies stressing preservation of genetic uniqueness and taxonomic integrity (Love Stowell *et al.* 2017) does not recognize that many small and at-risk populations:

> … will not be well-adapted to their current environment, due to fixation of deleterious alleles by random drift and changing local conditions due to human alteration of the environment, including global climate change". (Ralls *et al.* 2018: 2)

Another consideration is uncertainty in the causes of the numerical decline of the MNS Moose (NSD-NRR 2021b); there are several additional factors other than low genetic variability implicated as contributing to this decline. Thus, the concern remains that augmenting the MNS Moose population with individuals from other geographic locations might not have the desired effect of maintaining the population through improved genetic diversity and could simply result in the death of the introduced animals as a potential animal welfare issue (see Guideline 40 of the Canadian Council on Animal Care document on the Care and Use of Wildlife; https://www.ccac. ca/Documents/Standards/Guidelines/Wildlife.pdf). In summary, maintaining the natural gene flow between MNS and NB should be a high priority and, if necessary, genetic rescue—the introduction of individuals to supplement low levels of genetic variability and inbreeding depression—may be another effective conservation strategy to consider (Willi *et al.* 2022) while balancing the concerns noted above. As noted by Hedrick and Fredrickson (2010), one of their 10 guiding principles for genetic rescue is that the donor population should be closely related and ideally from a nearby area to minimize the likelihood of outbreeding depression. We suggest that Moose populations in New Brunswick, because of their high degree of genetic similarity, could be a source for genetic rescue of mainland Moose in Nova Scotia should the need arise. Although the data analyzed herein are likely indicative of neutral genetic variation, and so do not allow us to make any direct inferences about patterns of adaptive genetic variation in this region, using neutral variation for informing management strategies is still useful in lieu of alternative sources of information as part of a strategy to preserve as much genetic diversity as possible (García-Dorado and Caballero 2021).

## Author Contributions

## Acknowledgements

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