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Characterizing the demographic history and prion protein variation to infer susceptibility to chronic wasting disease in a naïve population of white-tailed deer (*Odocoileus virginianus*)

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Abstract

Assessments of the adaptive potential in natural populations are essential for understanding and predicting responses to environmental stressors like climate change and infectious disease. Species face a range of stressors in human-dominated landscapes, often with contrasting effects. White-tailed deer (*Odocoileus virginianus*; deer) are expanding in the northern part of their range following decreasing winter severity and increasing forage availability. Chronic wasting disease (CWD), a prion disease affecting deer, is likewise expanding and represents a major threat to deer and other cervids. We obtained tissue samples from free-ranging deer across their native range in Ontario, Canada, which has yet to detect CWD in wild populations. We used high-throughput sequencing to assess neutral genomic variation and variation in the prion protein gene (PRNP) that is partly responsible for the protein misfolding when deer contract CWD. Neutral variation revealed a high number of rare alleles and no population structure, and demographic models suggested a rapid historical population expansion. Allele frequencies of PRNP variants associated with CWD susceptibility and disease progression were evenly distributed across the landscape and consistent with deer populations not infected with CWD. We estimated the selection coefficient of CWD, with simulations showing an observable and rapid shift in PRNP allele frequencies that coincides with the start of a novel CWD outbreak. Sustained surveillance of genomic and PRNP variation can be a useful tool for guiding management practices, which is especially important for CWD-free regions where deer are managed for ecological and economic benefits.

KEYWORDS
Canadian wildlife, population genetics, prion, PRNP, RADseq, ungulate

1 | INTRODUCTION

Human-induced environmental change has caused widespread alterations to ecological and evolutionary processes (Harmon et al., 2009; Pecl et al., 2017). Climate change is expected to be the dominant driver of wildlife population declines and has been linked to broad-scale biodiversity losses, but regional responses are often nuanced and context dependent (e.g., Hashida et al., 2020; Taylor et al., 2017; White et al., 2017). For example, climate change in the Midwestern United States differentially favors the survival of two sympatric populations of ungulates with similar selection pressures and life-history traits, but differing densities (Escobar et al., 2019; Weiskopf et al., 2019). In such instances, intraspecific genetic diversity and adaptive potential are crucial for long-term population viability (Kardos & Shafer, 2018).

The emergence, spread, and persistence of infectious diseases in previously allopatric populations are facilitated by climate change and other anthropogenic activity (Aguirre, 2017; Morand & Walther, 2020; Price et al., 2016). The impacts of infectious disease on wildlife populations are of interest to managers, especially if the affected species holds economic or cultural value (Lambert et al., 2018; Weiskopf et al., 2019). Preventing and controlling diseases in free-ranging populations can, however, be complex and costly when they are both naïve to the infectious disease and faced with climate change and other anthropogenic activities (Herrera & Nunn, 2019; Miguel et al., 2020; Samuel et al., 2020). Selective pressures are increased under these circumstances and populations are forced to respond to multiple stressors simultaneously, or potentially face local extirpation (Fischer et al., 2020).

In wild populations, the functional adaptive response to ancient (Fedorov et al., 2020) and contemporary selection pressures (Mimura et al., 2016) causes changes to effective population size (N_e) and population demographics (McKnight et al., 2017). For example, the selective pressure exerted by novel infectious disease in naïve hosts can lead to sudden loss of genetic diversity (i.e., N_e) and sustained genetic bottlenecks (Phillips et al., 2020). When the adaptive potential of a population is diminished by low N_e, which is increasingly a problem in large mammals (e.g., Peart et al., 2020), genetic variation can be maintained in the short-term through successful dispersal to more suitable habitat or persistence within refugia (Chiocchio et al., 2019; Des Roches et al., 2019). In the worst case, low genetic variation can be further reduced by genetic drift and inbreeding and lead to local extirpation as species' no longer have an effective mechanism to respond to the changing environment (McKnight et al., 2017; Palkovacs et al., 2004). Characterizing neutral and functional genetic variation allows for both understanding and predicting adaptive potential, more specifically how wild populations will respond to the selective pressures of climate change, infectious disease, and anthropogenic activity (Funk et al., 2019).

White-tailed deer (*Odocoileus virginianus*; deer) are the most widely distributed and abundant ungulates in North America and hold significant economic and cultural value (Hewitt, 2011). The northern range of deer is primarily limited by snow but decreasing winter severity has allowed deer to expand northward beyond their historical range limits (Dawe & Boutin, 2016; Kennedy-Slaney et al., 2018). This expansion has implications for ecosystems as, for example, deer herbivory alters long-term regional habitat characteristics and plant communities (Frerker et al., 2017; Kroeger et al., 2020; Otsu et al., 2019). Further, deer are an important prey species and predator populations increasing in response to deer expansion has led to greater predation and apparent interspecific competition with other ungulates (Barber-Meyer & Mech, 2016; Latham et al., 2013). Consequently, northward expansions of deer are having profound impacts to ecosystems including facilitating infectious pathogen and disease spread (Averill et al., 2018; Ferretti & Mori, 2020).

A widespread threat to deer in North America is the highly infectious and fatal neurodegenerative prion disease called chronic wasting disease (CWD). CWD is the only prion disease known to infect captive and free-ranging species of cervids (family *Cervidae*) and has been reported in North and South America, Europe, and South Korea (Haley et al., 2019). With virtually no barriers to transmission and a lengthy infectious preclinical period, the local prevalence of CWD in North America has been measured to be as high as 50% and 82% in wild and captive populations, respectively (Miller et al., 2004; O'Rourke et al., 2004). Due to constraints on CWD surveillance, it is likely that the distribution and prevalence of CWD in wild populations are underestimated (Escobar et al., 2020). It is clear the frequency and occurrence of CWD have increased over time, in part driven by anthropogenic activities related to hunting and wildlife farming (Osterholm et al., 2019).

Despite CWD being fatal, there is inter-individual variation in susceptibility and clinical progression. Susceptibility and clinical progression are associated with non-synonymous and synonymous genetic variation in the functional prion protein gene (PRNP; Chafin et al., 2020; Güere et al., 2020). Single-nucleotide polymorphisms (SNPs) at nucleotide (nt) 60, nt153, nt285, nt286, nt555, and nt676 in deer PRNP have been associated with altered CWD susceptibility or pathogenic processes (Brandt et al., 2015, 2018; Johnson, Johnson et al., 2006; Wilson et al., 2009). The presence of CWD appears to affect population PRNP allele frequencies over space and time due to selection (Robinson et al., 2012); however, altered CWD susceptibility and pathogenic processes are clearly polygenic traits (Seabury et al., 2020) and disease spread is different in structured populations, which might require different wildlife management practices (Chafin et al., 2020). The efficacy of selection in the face of a novel pressure like CWD is dependent on N_e and common metrics to infer selection such as Tajima's *D* measure shifts in allele frequencies across the site frequency spectrum. However, the frequency and proportion of rare alleles are also sensitive to demographic processes (Messer et al., 2016; Platt et al., 2019), and population genetics theory predicts an excess of rare alleles in expanding populations (Gillespie, 2004).

Accordingly, at the genome level, we predicted an excess frequency of rare neutral and PRNP variants across our study region given Ontario's deer population is expanding (Kennedy-Slaney et al., 2018). Based on large-scale distribution changes and recent population trends in deer (Baldwin et al., 2000; Latch et al., 2009), we predicted we would

observe high neutral genomic diversity (N_e) and demographic population expansion, indicating increased gene flow and decreased population structure despite a heterogenous landscape. Since PRNP is not under selection by CWD given the region is disease-free, we predicted functional variation to resemble regions most recently exposed to CWD (or still disease-free). This is the first study to characterize PRNP genetic variation and population genomic structure of wild deer in Ontario, while also quantifying the ancient and contemporary demographic and selection processes shaping patterns of diversity.

2 | MATERIALS AND METHODS

2.1 | Study area and sample collection

We sampled white-tailed deer across Ontario, Canada (Figure 1). Ontario, Canada, reflects the northern leading edge of deer range in eastern North America (Kennedy-Slaney et al., 2018). The landscape of Ontario is heterogenous and environmental clines exist around the Great Lakes region. Ontario has not detected CWD in wild cervids, but CWD has been detected in farmed and captive cervids from virtually every jurisdiction bordering Ontario, with the province using a weighted surveillance approach to model CWD risk and strategically use resources for surveillance.

Between 2002 and 2018, retropharyngeal lymph nodes were opportunistically extracted from hunter-harvested deer across Ontario through the CWD surveillance program managed by the Ontario Ministry of Natural Resources and Forestry (OMNRF). Based on auxiliary data including year, sex, age-class, Mercator grid cell unit (GCU; 10 × 10 km), and wildlife management unit (WMU), we selected a subset of samples based on equal sampling of sex and geography. Northwestern and southern Ontario regions are geographically discontinuous for deer (Figure 1), samples were therefore assigned to northern Ontario or southern Ontario (Figure S1) for the purpose of analysis where sampling regions are compared. Preliminary analysis of data did not warrant separating southeastern and southwestern Ontario as per provincial management zones. Genomic DNA was extracted from deer samples using a silica-based DNA extraction kit for tissue following manufacturer protocol (Qiagen, Cat. No. 69506) and stored at −20°C. DNA quality was assessed by a spectrometer (NanoDrop 2000, Thermo Scientific) and by 2% agarose GelRed gel electrophoresis.

2.2 | Library preparation for PRNP genetic analysis

A 771 base pair (bp) region of the deer prion protein precursor (PRNP) gene was targeted and amplified using four degenerate primers (Table S1). Four replicate PCRs generated a 460 base pair
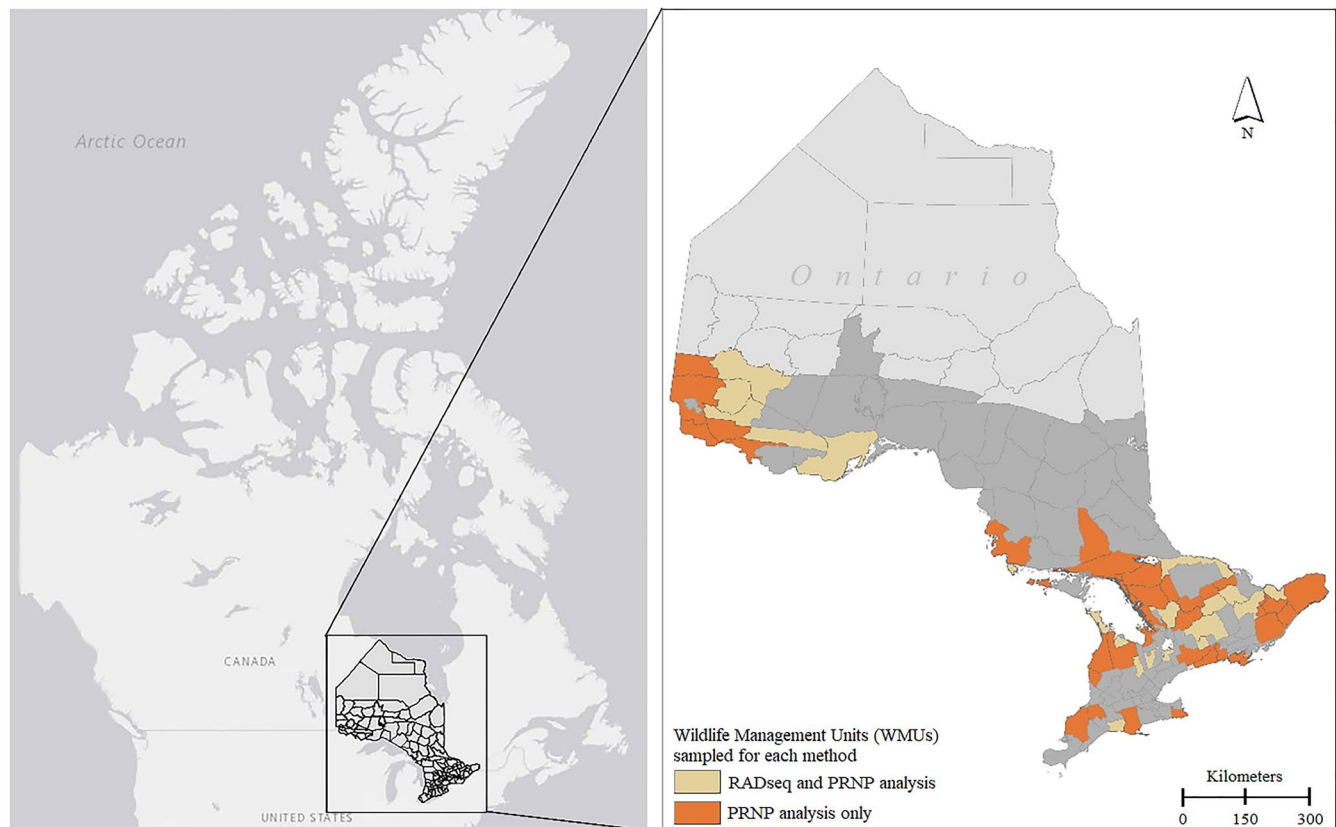
FIGURE 1 Distribution of the subsample of free-ranging white-tailed deer samples obtained between 2003 and 2018 by the Ontario Ministry of Natural Resources and Forestry (OMNRF) that were used for the reduced representation genome analysis ($n = 190$; cream) and the prion protein genetic analysis ($n = 631$; orange and cream). The natural distribution of free-ranging white-tailed deer is shown for Ontario with a darker shade of gray

Fragment 1 and a 580 base pair Fragment 2 (Table S2). In a 2:1 ratio of Fragment 1 to Fragment 2, respectively, amplified DNA was added for each individual and then indexed with standard Illumina multiplexing indices. Negative controls of UltraPure distilled water (Invitrogen, 1897011) were used for each 96-well plate. The library was purified of artifacts following manufacturer protocol for AMPure XP beads (Beckman Coulter, A63880) and validated with a TapeStation D1000 kit (Agilent, 5067-5582). The library was sequenced on an Illumina MiSeq platform at the University of Guelph Advanced Analysis Centre to generate 300 base pair (bp) pair-end reads for each sample.

2.3 | Library preparation for RADseq genomic analysis

Restriction site-associated DNA sequencing (RADseq) libraries were generated using an adapted protocol from Parchman et al. (2012) and Peterson et al. (2012) with Sbfl-HF and MseI restriction enzymes. Samples were incubated, digested overnight, and heat-inactivated in 96-well plates (Table S3). Negative controls of UltraPure distilled water (Invitrogen, 1897011) were used for each 96-well plate. Restriction digested DNA was combined with 7 μl of ligation mixture and 3 μl of one of the 24 available Sbfl adapters (1.0 μM). Adapters were ligated at 16°C for 3 h. DNA fragments were purified of artifacts following manufacturer protocol for AMPure XP beads (Beckman Coulter, A63880). Adapter-ligated fragments were amplified in four separate 10 μl reactions that incorporated barcodes. Reaction conditions and primers are shown for ligation mixture and PCR in Tables S4 and S5, respectively. Samples were pooled and purification was performed following manufacturer protocol for QIAquick PCR Purification kit (Qiagen, 28106) for a final elution to 42 μl. Size selection between 450 bp to 700 bp was performed on 80 μl replicates of purified library and gel purification was performed following manufacturer protocol for QIAquick Gel Extraction kit (Qiagen, 28706) for a final elution to 60 μl. The purified final library was validated with a TapeStation D1000 kit (Agilent, 5067-5582). The libraries were sequenced at The Centre for Applied Genomics (TCAG) in The Hospital for Sick Children (SickKids) on an Illumina HiSeq 2500 to produce 2×126 base pair paired-end reads.

2.4 | Bioinformatic pipeline and data analysis I – PRNP gene

The quality of reads was assessed using FastQC (Babraham Institute; v0.11.8). Samples were excluded if at least one file in the pair-end files for a sample was less than 1 kB in size or failed to pass quality standards. A novel command line-based pipeline was developed to assemble and genotype PRNP (accessible at www.gitlab.com/WiDGeT_TrentU/graduate_theses). Our workflow integrated Pullseq v1.0.2, BWA v0.7.17, SAMtools; v1.9, BCFtools v1.9, and VCFtools v0.1.16-15. Briefly, for each sample, the pipeline

extracted relevant reads based on the presence of primer sequence, mapped the extracted reads to a 771 bp PRNP gene reference sequence. We generated a consensus sequence and called single-nucleotide polymorphisms (SNPs). SNP calls were limited to positions where there was a minimum read depth of 30 and mapping quality score of at least 30. Sanger sequencing of a subset of samples and their a priori called variants were used to validate the bioinformatic pipeline.

The presence of asparagine (N) at aa138 (nt413A) indicates amplification of the pseudogene (Brandt et al., 2015); we therefore filtered out all sequences with this site. SNPs with a total frequency of occurrence of 1% or less were excluded from the analysis. A two-sided Fisher's Exact Test was conducted on minor allele counts from either northern or southern sampling regions at four well-studied positions in the deer PRNP gene associated with CWD: nucleotide (nt) 60, nt285, nt286, and nt676. Synonymous and non-synonymous sites were identified using MEGA X v10.0.5. Haplotypes were estimated from unphased sequences with PHASE v2.1.1 using a Markov chain Monte Carlo (MCMC) sampling approach with a minimum of 100,000 steps, with a discarded burn-in of 10,000, and samples were drawn every 100 MCMC steps. Five repetitions were performed to verify consistent frequencies of haplotype assignment (Brandt et al., 2018). Haplotypes with a frequency of less than 1% were removed. The genotype, frequencies, and estimated standard deviations of the remaining haplotypes were analyzed as a 2×2 contingency table by sampling region.

2.5 | Bioinformatic pipeline and data analysis II – RADseq

Fastq files were demultiplexed using process_radtags within the Stacks v2.3 module. Parameters within process_radtags included the removal of any read with an uncalled base and the discarding of reads with low-quality scores. The demultiplexed sample files were aligned against the deer genome (Genome Accession JAAVWD000000000) using BWA with samtools used to sort, merge and compress BAM files. The referenced-based approaches on gstacks and populations program within STACKS produced a variant call format (VCF) file with the restrictions that the minimum percentage of individuals in a population required to process a locus for that population was 90%. The VCF was filtered using VCFtools to only include reads with a minimum read depth of 20. Population statistics, including F_{IS}, observed and estimated homozygosity, and nucleotide diversity were calculated using the 'populations' module. To analyze sources of variation, we generated a principal component analysis (PCA) using the R v3.6.1 package adegenet v2.1.3. A linear regression was run on principal component (PC) 1 and PC2 scores against latitude and longitude. We estimated F_{ST} between north and south using StAMPP v1.6.1. Population structure was detected using successive K-means clustering and a discriminant analysis of principal components (DAPC) available in adegenet (Jombart et al., 2010).

2.6 | Demographic analysis and estimate of effective population size

The final VCF was converted into 1D site frequency spectrum (SFS) for all of Ontario and northern vs and southern designations, respectively, using vcf2dadi.py with projections for the SFS estimated in easySFS. We applied a diffusion-based approach to demographic inference through the Diffusion Approximation for Demographic Inference (δaδi) tool by Gutenkunst et al. (2009). Nine 1D models were assessed for Ontario as a single population. The optimum model was selected as the lowest optimized log-likelihood of all successfully run models. δaδi was also used to estimate the following summary statistics for the province: Watterson Theta (θ), Tajima's D, and the number of segregating sites. Using the mutation rate (μ) per site per generation of a closely related species (*Rangifer tarandus* from Chen et al., 2019), total number of sites (L), and the parameters estimated in the optimum model selected from δaδi, we estimated the ancestral effective population size as $N_a = \theta/4\ \mu l$.

2.7 | Estimation of selection on PRNP and allele frequency projections for a naïve population

We estimated the selection coefficient (s) at two polymorphic sites (nt285A to C and nt286A to G) using the approach of Thompson et al. (2019) which accounts for number of generations and different expression modes (i.e., recessive, dominant, and codominant). Strength of selection against the less resistant phenotype [i.e., the homozygous common allele (s_{AA})] can be estimated by calculating values of s that explained the estimated change in allele frequencies between positive and negative animals from the same region. Here we used starting (negative CWD) allele frequencies from Wilson et al. (2009) and Kelly et al. (2008) and estimated s over n generations the equation:

$$p\prime = (1 - s_{AA})\,p^2 + (1 - s_{AB})\,p(1 - p) / (1 - s_{AA})\,p^2 + (1 - s_{AB})\,2p(1 - p) + (1 - s_{BB})(1 - p)^2)$$

taken from Charlesworth and Charlesworth (2010). To account for uncertainty in time and allele frequency estimates we ran 100 iterations with n ranging from 2 to 25 generations (~4–50 deer years) and positive and negative allele frequencies ($\pm1\%$) for those reported from both Wilson et al. (2009) and Kelly et al. (2008). Using our estimated allele frequencies for Ontario, we then projected each allele frequencies into the next 25 generations using our estimated selection coefficients, and the estimated s coefficients of 0.0103 and 0.074 from Robinson et al. (2012). Calculations were conducted under three relative fitness scenarios as per Thompson et al. (2019).

3 | RESULTS

3.1 | PRNP genetic analysis

A total of 631 Ontario deer samples were included in the PRNP genetic analysis (Figure 2). Nineteen SNPs were detected after filtering (Table 1), with eight being non-synonymous substitutions. Six of the detected variants in the PRNP gene have been linked to CWD susceptibility or clinical progression, these include nt60, nt153, nt285, nt286, nt555, and nt676. A two-sided Fisher's Exact test on the major and minor allele counts at the four important, arguably the most studied, CWD-linked loci (nt60, nt285, nt286, and nt676) conducted between Northern and Southern Ontario indicated that there was only a small difference in frequency ($p < 0.05$) at nt676 (Table S6), collectively showing that PRNP variants are more-or-less distributed similarly across the province.

There were 102 unique haplotypes with a count of at least one, with 12 haplotypes having a frequency greater than 1% (Table S7). The two most common haplotypes, Haplotype 3 ($f = 0.23$) and Haplotype 1 ($f = 0.12$) did not include any non-synonymous substitutions. Haplotype A ($f = 0.30$) and Haplotype B ($f = 0.25$) reported by Brandt et al. (2015, 2018) from northern Illinois were also detected as Haplotype 16 ($f = 0.09$) and Haplotype 7 ($f = 0.05$), respectively. The same Haplotype A ($f = 0.15$) and Haplotype B ($f = 0.23$) were reported by Chafin et al. (2020) in deer from Arkansas, USA.

3.2 | RADseq genomic analysis

A total of 235 Ontario deer samples were sequenced in ddRADseq libraries. Following quality control and quality assessment, including FastQC and line counts of demultiplexed files, 190 samples remained for downstream analysis (Figure 1). Estimated population diversity statistics are summarized in Table 2. The PCA clearly separated northern and southern Ontario along PC1 (Figure 3). A linear regression revealed that PC1 was strongly associated with longitude ($\beta = -0.79$; adjusted $R^2 = 0.77$; p-value <0.01), a pattern consistent with the northward expansion of deer. PC2 was beset correlated with latitude ($\beta = -0.82$; adjusted $R^2 = 0.157$; p-value = <0.01). However, population structure between northern and southern Ontario was weak ($F_{ST} = 0.02$). A BIC based on the K-cluster analysis also indicated that the most optimum number of clusters was 1 (Figure S2), suggesting a high degree of genetic homogeneity across Ontario.

The optimum 1D demographic model for Ontario was the BOTTLEGROWTH model, which models an instantaneous size change followed by exponential change (Table S8); here, there is large historical growth in population, followed by a more recent and moderate decline (Figure 4). From the 1D site frequency spectrum, the mean Tajima's D was estimated to be −2.126 consistent with a population expansion after a bottleneck. Using the calculated θ, we estimated N_a to be ~20,000: this would place the timing of the population change (Tc) measured in 2N$_a$ generations around the onset of the last glacial maximum (Figure 4).

3.3 | PRNP selection and projection

We estimated the selection coefficients to be 0.08 (±0.06) and 0.11 (±0.07) for nt285 and nt286 under a dominance model. All simulated projections with our s values showed a rapid shift in allele
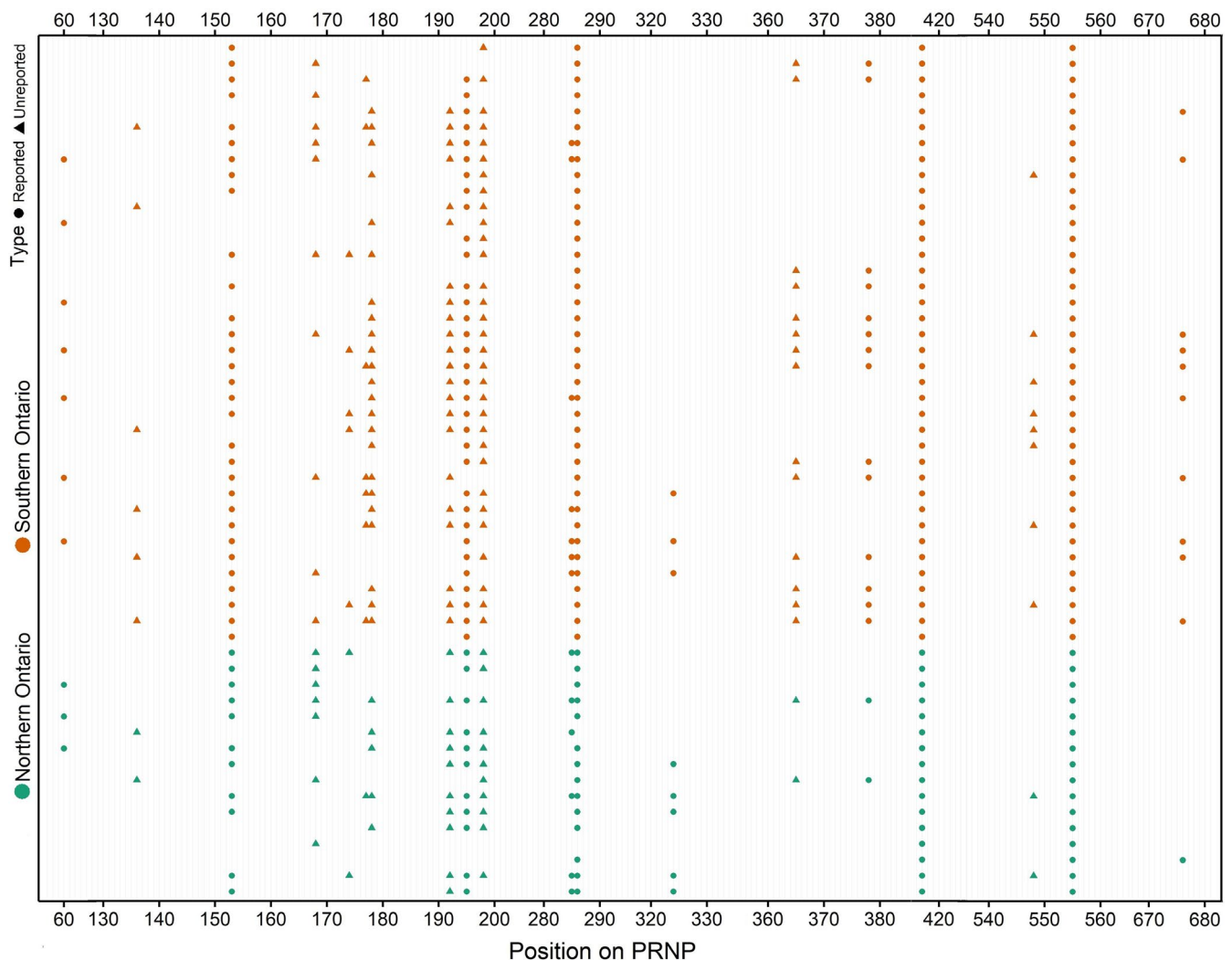
FIGURE 2 A 771 bp region of the white-tailed deer prion protein gene was analyzed from free-ranging white-tailed deer in Ontario, Canada (*n* = 631). The stacked polymorphisms across 19 variable loci were organized by broad management in Ontario and are shown. Circles indicate loci previously described as variable in the white-tailed deer prion protein gene. Triangles indicate novel variable loci in the white-tailed deer prion protein gene

frequencies (Figure 5); the majority of simulated trajectories did not overlap with the lower *s* coefficient (0.01) previously reported by Robinson et al. (2012), but were consistent with their higher coefficient of 0.074.

4 | DISCUSSION

White-tailed deer in North America are intensively managed for hunter harvest and are expanding their range northward due to climate change. Our genome assessment showed high levels of variation and homogeneity in Ontario, with demographic models showing evidence of a massive species-wide population expansion. The frequency and occurrence of CWD infection in captive and free-ranging deer are likewise increasing (Osterholm et al., 2019; Rivera et al., 2019), but transmission and spread in free-ranging populations

are still poorly understood (Potapov et al., 2016). In Ontario, we observed multiple novel PRNP alleles, with the frequencies of known variants differing from areas currently infected with CWD (or where CWD is endemic), including western Canada (Table S9; Brandt et al., 2015, 2018; Chafin et al., 2020; Kelly et al., 2008; Wilson et al., 2009). Collectively, our functional and neutral genomic data are consistent with CWD not being present in Ontario.

The emergence, transmission, and persistence of highly infectious diseases in healthy populations are often facilitated by climate change and exacerbated in areas with intense anthropogenic activity (McKnight et al., 2017; Rizzoli et al., 2019). The introduction and re-introduction of infectious diseases often result in rapid local population declines, reducing species' adaptive potential and generating substantial economic losses (Belant & Deese, 2010; Escobar et al., 2019). In Ontario deer, however, we observed an excess of rare alleles with no evidence of strong population

TABLE 1 Allele and amino acid frequencies for reference/alternate alleles at Ontario white-tailed deer ($n = 631$) prion protein gene

Locus	Major/Minor allele(s)	Codon	Amino acid	Major frequency	Minor frequency
60	C/T	20	D/-	0.9794	0.0206
136[a]	A/G	46	S/G	0.9873	0.0127
153	C/T	51	R/-	0.8605	0.1395
168[a]	A/G	56	G/-	0.9635	0.0365
174[a]	T/G	58	G/-	0.9842	0.0158
177[a]	C/G	59	G/-	0.9873	0.0127
178[a]	T/G	60	W/G	0.9160	0.0840
192[a]	T/G	64	H/Q	0.8653	0.1347
195[a]	A/G, T	65	G/-	0.7147	0.2853
198[a]	T/G	66	G/-	0.7021	0.2979
285	A/C	95	Q/H	0.9699	0.0301
286	G/A	96	G/S	0.6609	0.3391
324	A/G	108	P/-	0.9794	0.0206
365[a]	G/T	122	G/V	0.9651	0.0349
378	G/A	126	G/-	0.9651	0.0349
417	G/A	139	R/-	0.1696	0.8304
548[a]	T/A	183	V/D	0.9778	0.0222
555	C/T	185	I/-	0.4120	0.5880
676	C/A	226	Q/K	0.9620	0.0380

[a]Indicates novel positions.

TABLE 2 Genome-wide population summary statistics including breakdown of sites, number of individuals, nucleotide diversity estimate (π), individual genetic variance (I) relative to the subpopulation genetic variance (F_{IS}), and the observed heterozygosity of white-tailed deer in Ontario

Group	Ontario	Northern	Southern
Number individuals	182 ± 21	57 ± 3	125 ± 15
Total sites	13,439,671	14,042,841	12,885,176
Polymorphic sites	165,254	139,582	146,036
π	5.0×10^{-4}	6.4×10^{-4}	5.7×10^{-4}
Observed heterozygosity	4.7×10^{-4}	6.1×10^{-4}	5.5×10^{-4}
F_{IS}	5.7×10^{-4}	3.9×10^{-4}	4.2×10^{-4}

structure, including in southern Ontario where an environmental cline exists around lake systems. An excess of rare alleles and high N_e and absent population structure might provide the means for effective adaptation to selective pressures, including climate change, infectious disease, and human activity.

4.1 | Linking population demographics to functional genes

We assessed contemporary and historical gene flow by examining neutral genomic variation across tens of thousands of loci from deer across the province on Ontario. The data were consistent with a large expanding population with a high level of genomic diversity and an excess of rare alleles. These genomic patterns are also consistent with population simulations of responses to climate change (Kennedy-Slaney et al., 2018) and offer some clues as to the

potential adaptive response were CWD to arrive. Specifically, two important features of the genomic data are noteworthy: the high number of rare alleles and the limited population structure across Ontario.

Demographic processes and life-history strategies influence the proportion of rare alleles, which are important to the adaptive process but are sensitive to recent evolutionary processes (Peart et al., 2020). Evidence suggests the accumulation of rare alleles is independent of taxa, but adaptation appears limited in low-diversity taxa (e.g., primates; Rousselle et al., 2020). The deer genomic data evaluated here are consistent with high adaptive potential; specifically, we calculated an N_a to be ~20,000, with the expansion estimated to be 100X suggestive of a large species-wide N_e (derived either from π in Table 2 or the demographic model in Figure 4). Studies of populations infected with CWD often demonstrate a lack of allelic diversity at the PRNP gene, which is thought to be due to CWD-driven selection positively selecting for functionally

FIGURE 3 A plot of PC1 and PC2 from the principal component analysis (PCA) on the reduced representation white-tailed deer genome. PC1 and PC2 were able to explain a total of 4.3% of the genomic variation observed. Gray circles represent scores from samples in northern Ontario. Orange circles represent scores from samples in southern Ontario
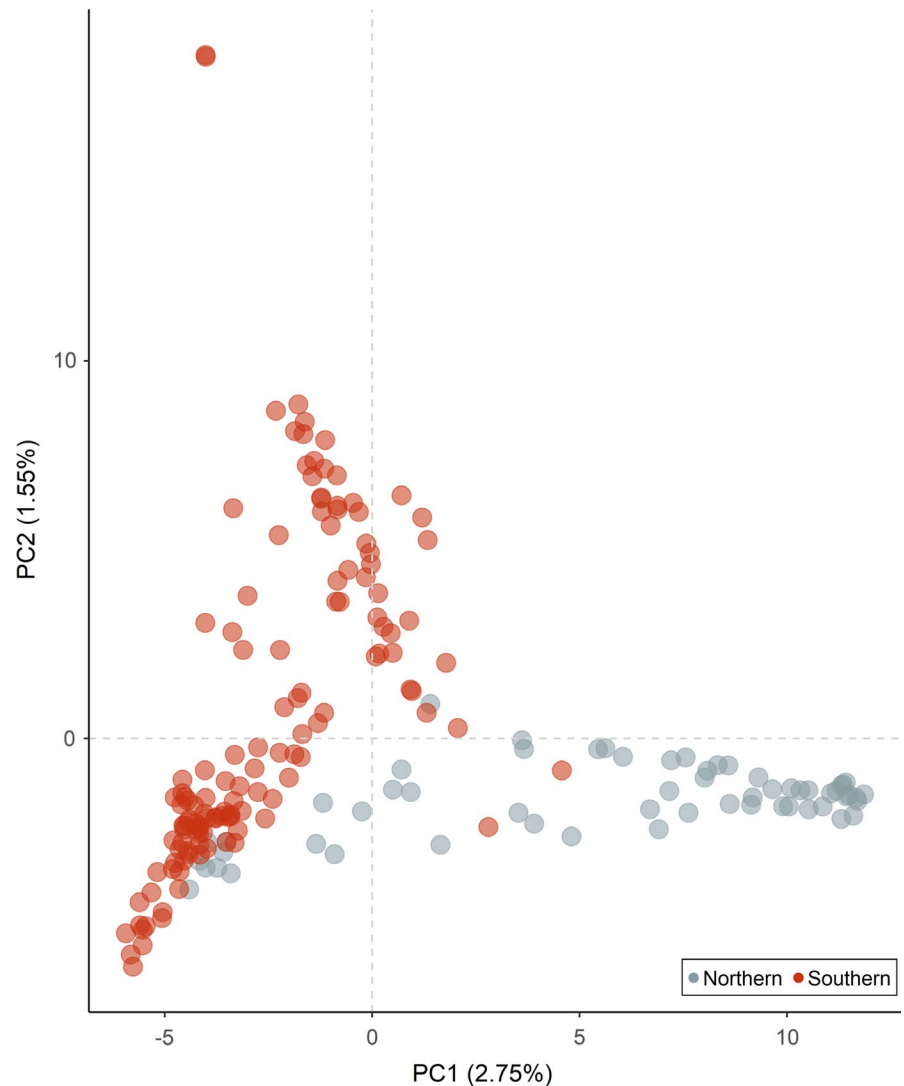
relevant alleles (Haley et al., 2019). We also found a high frequency of rare PRNP alleles in our naïve population which differs from areas currently infected or where CWD is endemic, including western Canada (Brandt et al., 2015, 2018; Johnson, Johnson et al., 2006; Kelly et al., 2008; Wilson et al., 2009). Furthermore, infectious diseases can cause population fragmentation (i.e., structure), demographic changes, and genetic isolation (McKnight et al., 2017). A pre-infection presence of a large proportion of rare alleles in both functional and neutral genes, as observed in Ontario, supports surveillance data showing no CWD, which might bode well for a long-term adaptive response to CWD infection and other stressors. Importantly, even low selection coefficients should alter allele frequencies in a detectable manner (Figure 5). However, it is not clear how this standing genetic variation compares to infected populations prior to the arrival of CWD, creating some uncertainty in the potential adaptive response, recognizing that loci outside PRNP are also involved (Seabury et al., 2020).

We observed no evidence of strong population structure despite the heterogeneous landscape observed in Ontario, but there is a clear latitudinal cline in allele frequencies. This suggests random mating is largely occurring at a regional level in spite of substantial environmental changes including intense agricultural practices, substantial urbanization of the landscape, and climate change (Patton et al., 2018; Schulte et al., 2007; Walter et al., 2009). We might therefore expect that the spread of advantageous rare alleles under selection may not be limited in the province by the same barriers experienced in structured populations. At the same time, we might also expect the homogenous population to facilitate the spread of CWD if anthropogenic activity were to introduce the disease into the focal population (Escobar et al., 2020). The movement of infected wildlife might also pose a potential risk to human health at the wildlife–human–livestock interface as CWD can cross species barriers (Igel-Egalon et al., 2020). Sustained monitoring across CWD-free regions where deer are managed for sustainability or where food security is threatened should continue but consider population-level responses to climate change (i.e., Kennedy-Slaney et al., 2018) while integrating genetic information beyond PRNP allele frequencies.
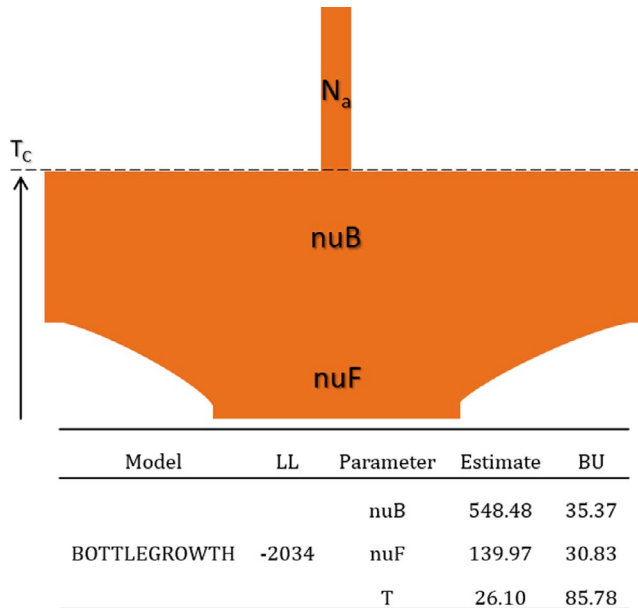
FIGURE 4 Demographic parameter estimates from δaδi for the optimal 1D model for white-tailed deer in Ontario. Parameter estimates are the ancient population size (Na), the ratio of population size after instantaneous change to ancient population size (nuB); the ratio of contemporary to ancient population size (nuF); and the time in the past at which instantaneous change happened and growth began (TC; in units of 2*Na generations). Included are the optimized log-likelihood (LL) and bootstrap uncertainties (BU)

Model	LL	Parameter	Estimate	BU
		nuB	548.48	35.37
BOTTLEGROWTH	-2034	nuF	139.97	30.83
		T	26.10	85.78

5 | CONCLUSION

We gauged the adaptive potential of CWD naïve deer in Ontario, Canada by assessing functional and neutral genetic diversity. The genome-wide data were consistent with a large expanding population with a high neutral genomic diversity, no population structure, and an excess of rare alleles, which is also consistent with population and climate models (Kennedy-Slaney et al., 2018). Voluntary hunter harvest-based surveillance for CWD will likely be able to detect the introduction of CWD in a naïve population; however, we expect that there will be a lag in detection when prevalence is low since low densities likely limit transmission (Gagnier et al., 2020). A lag in detection will likely permit the establishment of CWD and, over time, eradication becomes nearly impossible and costs are high (Mysterud & Rolandsen, 2018). Targeted temporal monitoring of variation in PRNP across CWD-free regions could be a detection tool as our simulations suggest detectable shifts should occur with the arrival of the disease. Monitoring frequency shifts at other geographic locations (and other genes) could be easily integrated into our simulations and might identify additional variants of disease relevance. Overall, by combining demographic patterns and genotypes with current risk models, managers could improve risk-based detection efforts and facilitate a more effective resource deployment plan as the disease alters the population.
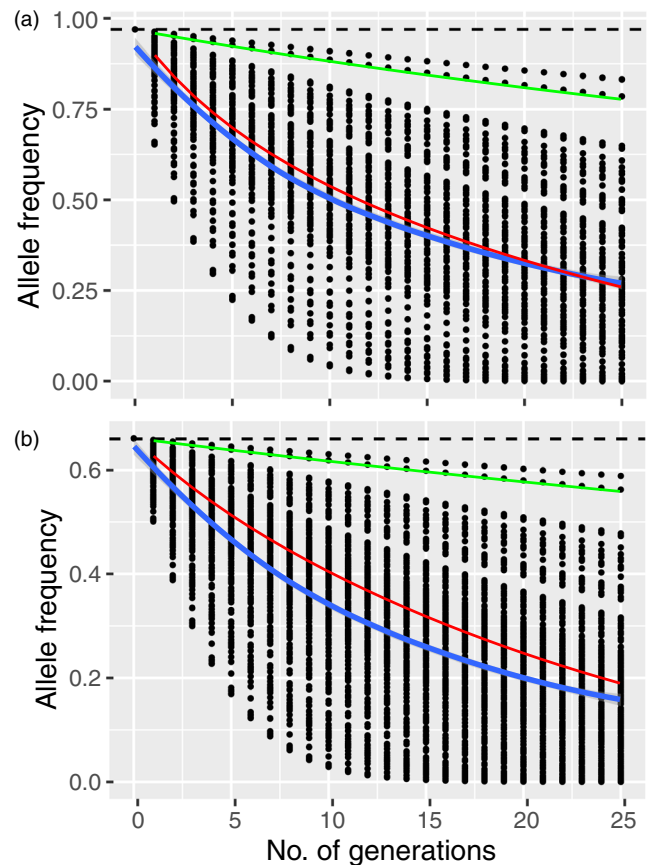
FIGURE 5 Simulated allele frequency projections for nucleotide positions (a) 285 A/C and (b) 743286 G/A in the PRNP gene under selection. Green and red lines are the selection coefficients provided by Robinson et al. (2012), while the black dots are those from our simulations, with blue line representing the mean selection coefficient

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The raw data are accessible on the Sequence Read Archive (PRJNA565222 and SUB8436966). All scripts and VCF files are accessible at www.gitlab.com/WiDGeT_TrentU/graduate_theses.

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REFERENCES

Aguirre, A. A. (2017). Changing patterns of emerging zoonotic diseases in wildlife, domestic animals, and humans linked to biodiversity loss and globalization. *ILAR Journal*, 58(3), 315–318. https://doi.org/10.1093/ilar/ilx035

Averill, K. M., Mortensen, D. A., Smithwick, E. A. H., Kalisz, S., McShea, W. J., Bourg, N. A., Parker, J. D., Royo, A. A., Abrams, M. D., Apsley, D. K., Blossey, B., Boucher, D. H., Caraher, K. L., DiTommaso, A., Johnson, S. E., Masson, R., & Nuzzo, V. A. (2018). A regional assessment of white-tailed deer effects on plant invasion. *AoB PLANTS*, 10(1), plx047. https://doi.org/10.1093/aobpla/plx047

Baldwin, D. J. B., Desloges, J. R., & Band, L. E. (2000). Physical geography of Ontario. In A. H. Perera, D. L. Euler, & I. D. Thompson (Eds.), *Ecology of a managed terrestrial landscape: Patterns and processes of forest landscapes in Ontario* (1, pp. 141–162). Ontario Ministry of Natural Resources, and UBC Press.

Barber-Meyer, S. M., & Mech, L. D. (2016). White-tailed deer (*Odocoileus virginianus*) subsidize gray wolves (*Canis lupus*) during a moose (*Alces americanus*) decline: a case of apparent competition? *Canadian Field Naturalist*, 130(4), 308–314. https://doi.org/10.22621/cfn.v130i4.1924

Belant, J. L., & Deese, A. R. (2010). Importance of wildlife disease surveillance. *Human-Wildlife Interaction*, 4(2), 165–169.

Brandt, A. L., Green, M. L., Ishida, Y., Roca, A. L., Novakofski, J., & Mateus-Pinilla, N. E. (2018). Influence of the geographic distribution of prion protein gene sequence variation on patterns of chronic wasting disease spread in white-tailed deer (*Odocoileus virginianus*). *Prion*, 12(3–4), 204–215. https://doi.org/10.1080/19336896.2018.1474671

Brandt, A. L., Kelly, A. C., Green, M. L., Shelton, P., Novakofski, J., & Mateus-Pinilla, N. E. (2015). Prion protein gene sequence and chronic wasting disease susceptibility in white-tailed deer (*Odocoileus virginianus*). *Prion*, 9(6), 449–462. https://doi.org/10.1080/19336896.2015.1115179

Chafin, T. K., Douglas, M. R., Martin, B. T., Zbinden, Z. D., Middaugh, C. R., Ballard, J. R., Gray, M. C., White, D., & Douglas, M. E. (2020). Age structuring and spatial heterogeneity in prion protein gene (PRNP) polymorphism in white-tailed deer. *Prion*, 14, 238–248. https://doi.org/10.1101/2020.07.15.205039v2

Charlesworth, B., & Charlesworth, D. (2010). *Elements of evolutionary genetics*. Roberts & Company. https://doi.org/10.1017/S001667231000042X

Chen, L., Qiu, Q., Jiang, Y. U., Wang, K., Lin, Z., Li, Z., Bibi, F., Yang, Y., Wang, J., Nie, W., Su, W., Liu, G., Li, Q., Fu, W., Pan, X., Liu, C., Yang, J., Zhang, C., Yin, Y., ... Wang, W. (2019). Large-scale ruminant genome sequencing provides insights into their evolution and distinct traits. *Science*, 364(6446), eaav6202. https://doi.org/10.1126/science.aav6202

Chiocchio, A., Colangelo, P., Aloise, G., Amori, G., Bertolino, S., Bisconti, R., & Canestrelli, D. (2019). Population genetic structure of the bank vole *Myodes glareolus* within its glacial refugium in peninsular Italy. *Journal of Zoological Systematics and Evolutionary Research*, 57(4), 959–969. https://doi.org/10.1111/jzs.12289

Dawe, K. L., & Boutin, S. (2016). Climate change is the primary driver of white-tailed deer (*Odocoileus virginianus*) range expansion at the northern extent of its range; land use is secondary. *Ecology and Evolution*, 6(18), 6435–6451. https://doi.org/10.1002/ece3.2316

Des Roches, S., Bell, M. A., & Palkovacs, E. P. (2019). Climate-driven habitat change causes evolution in three spine Stickleback. *Global Change Biology*, 26(2), 597–606. https://doi.org/10.1111/gcb.14892

Escobar, L. E., Moen, R., Craft, M. E., & VanderWaal, K. L. (2019). Mapping parasite transmission risk from white-tailed deer to a declining moose population. *European Journal of Wildlife Research*, 65. 1–11. https://doi.org/10.1007/s10344-019-1297-z

Escobar, L. E., Pritzkow, S., Winter, S. N., Grear, D. A., Kirchgessner, M. S., Dominquez-Villegas, E., Machado, G., Peterson, A. T., & Soto, C. (2020). The ecology of chronic wasting disease in wildlife. *Biological Reviews*, 95(2), 393–408. https://doi.org/10.1111/brv.12568

Fedorov, V. B., Trucchi, E., Goropashnaya, A. V., Waltari, E., Whidden, S. E., & Stenseth, N. C. (2020). Impact of past climate warming on genomic diversity and demographic history of collared lemmings across the Eurasian Arctic. *Proceedings of the National Academy of Sciences of the United States of America*, 117(6), 3026–3033. https://doi.org/10.1073/pnas.1913596117

Ferretti, F., & Mori, E. (2020). Displacement interference between wild ungulate species: does it occur? *Ethology Ecology & Evolution*, 32(1), 2–15. https://doi.org/10.1080/03949370.2019.1680447

Fischer, K., Kreyling, J., Beaulieu, M., Beil, I., Bog, M., Bonte, D., Holm, S., Knoblauch, S., Koch, D., Muffler, L., Mouginot, P., Paulinich, M., Scheepens, J. F., Schiemann, R., Schmeddes, J., Schnittler, M., Uhl, G., van der Maaten-Theunissen, M., Weier, J. M., ... Gienapp, P. (2020). Species-specific effects of thermal stress on the expression of genetic variation across a diverse group of plant and animal taxa under experimental conditions. *Heredity*, 126, 23–37. https://doi.org/10.1038/s41437-020-0338-4

Frerker, K., Sabo, A., & Waller, D. (2017). Correction: long-term regional shifts in plant community composition are largely explained by local deer impact experiments. *PLoS One*, 12(9), e0185037. https://doi.org/10.1371/journal.pone.0185037

Funk, W. C., Forester, B. R., Converse, S. J., Darst, C., & Morey, S. (2019). Improving conservation policy with genomics: a guide to integrating adaptive potential into U.S. Endangered Species Act decisions for conservation practitioners and geneticists. *Conservation Genetics*, 20, 115–134. https://doi.org/10.1007/s10592-018-1096-1

Gagnier, M., Laurion, I., & DeNicola, A. J. (2020). Control and surveillance operations to prevent chronic wasting disease establishment in free-ranging white-tailed deer in Québec, Canada. *Animals*, 10, 283. https://doi.org/10.3390/ani10020283

Gillespie, J. H. (2004). *Population genetics: A concise guide* (2nd ed.). The Johns Hopkins University Press.

Gutenkunst, R. N., Hernandez, R. D., Williamson, S. H., & Bustamante, C. D. (2009). Inferring the joint demographic history of multiple populations from multidimensional SNP frequency data. *PLoS Genetics*, 5, e1000695.

Güere, M. E., Våge, J., Tharaldsen, H., Benestad, S. L., Vikøren, T., Madslien, K., Hopp, P., Rolandsen, C. M., Røed, K. H., & Tranulis, M. A. (2020). Chronic wasting disease associated with prion protein gene (PRNP) variation in Norwegian wild reindeer (*Rangifer tarandus*). *Prion*, 14(1), 1–10. https://doi.org/10.1080/19336896.2019.1702446

Haley, N. J., Merrett, K., Buros Stein, A., Simpson, D., Carlson, A., Mitchell, G., Staskevicius, A., Nichols, T., Lehmkuhl, A. D., & Thomsen, B. V. (2019). Estimating relative CWD susceptibility and disease progression in farmed white-tailed deer with rare PRNP alleles. *PLoS One*, 14(12), e0224342. https://doi.org/10.1371/journal.pone.0224342

Harmon, J. P., Moran, N. A., & Ives, A. R. (2009). Species response to environmental change: impacts of food web interactions and evolution. *Science*, 323(5919), 1347–1350. https://doi.org/10.1126/science.1167396

Hashida, Y., Withey, J., Lewis, D. J., Newman, T., & Kline, J. D. (2020). Anticipating changes in wildlife habitat induced by private forest owners' adaptation to climate change and carbon policy. *PLoS One*, 15(4), e0230525. https://doi.org/10.1371/journal.pone.0230525

Herrera, J., & Nunn, C. L. (2019). Behavioural ecology and infectious disease: implications for conservation of biodiversity. *Philosophical Transactions of the Royal Society of London Series B Biological Sciences*, 374(1781), 20180054. https://doi.org/10.1098/rstb.2018.0054

Hewitt, D. G. (2011). *Biology and management of white-tailed deer* (ed.). CRC Press.

Igel-Egalon, A., Laferrière, F., Tixador, P., Moudjou, M., Herzog, L., Reine, F., Torres, J. M., Laude, H., Rezaei, H., & Béringue, V. (2020). Crossing species barriers relies on structurally distinct prion assemblies and their complementation. *Molecular Neurobiology*, *57*(6), 2572–2587. https://doi.org/10.1007/s12035-020-01897-3

Johnson, C., Johnson, J., Vanderloo, J. P., Keane, D., Aiken, J. M., & McKenzie, D. (2006). Prion protein polymorphisms in white-tailed deer influence susceptibility to chronic wasting disease. *The Journal of General Virology*, *87*(7), 2109–2114. https://doi.org/10.1099/vir.0.81615-0

Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics*, *11*, 94. https://doi.org/10.1186/1471-2156-11-94

Kardos, M., & Shafer, A. B. A. (2018). The peril of gene-targeted conservation. *Trends in Ecology and Evolution*, *33*(11), 827–839. https://doi.org/10.1016/j.tree.2018.08.011

Kelly, A. C., Mateus-Pinilla, N. E., Diffendorfer, J., Jewell, E., Ruiz, M. O., Killefer, J., Shelton, P., Beissel, T., & Novakofski, J. (2008). Prion sequence polymorphisms and chronic wasting disease resistance in Illinois white-tailed deer (*Odocoileus virginianus*). *Prion*, *2*(1), 28–36. https://doi.org/10.4161/pri.2.1.6321

Kennedy-Slaney, L., Bowman, J., Walpole, A. A., & Pond, B. A. (2018). Northward bound: the distribution of white-tailed deer in Ontario under a changing climate. *Wildlife Research*, *45*(3), 220–228. https://doi.org/10.1071/WR17106

Kroeger, A. J., Moorman, C. E., Lashley, M. A., Chitwood, M. C., Harper, C. A., & DePerno, C. S. (2020). White-tailed deer use of overstory hardwoods in longleaf pine woodlands. *Forest Ecology and Management*, *464*, 118046.

Lambert, S., Ezanno, P., Garel, M., & Gilot-Fromont, E. (2018). Demographic stochasticity drives epidemiological patterns in wildlife with implications for diseases and population management. *Scientific Reports*, *8*, 16846. https://doi.org/10.1038/s41598-018-34623-0

Latch, E. K., Heffelfinger, J. R., Fike, J. A., & Rhodes, O. E. (2009). Species-wide phylogeography of North American mule deer (*Odocoileus hemionus*): cryptic glacial refugia and postglacial recolonization. *Molecular Ecology*, *18*(8), 1730–1745. https://doi.org/10.1111/j.1365-294X.2009.04153.x

Latham, A. D. M., Latham, M. C., Knopff, K. H., Hebblewhite, M., & Bouting, S. (2013). Wolves, white-tailed deer, and beaver: implications of seasonal prey switching for woodland caribou declines. *Ecography*, *36*(12), 1276–1290. https://doi.org/10.1111/j.1600-0587.2013.00035.x

McKnight, D. T., Schwarzkopf, L., Alford, R. A., Bower, D. S., & Zenger, K. R. (2017). Effects of emerging infectious disease on host population genetics: a review. *Conservation Genetics*, *18*, 1235–1245. https://doi.org/10.1007/s10592-017-0974-2

Messer, P. W., Ellner, S. P., & Hairson, N. G. Jr (2016). Can population genetics adapt to rapid evolution? *Trends in Genetics*, *32*(7), 408–418. https://doi.org/10.1016/j.tig.2016.04.005

Miguel, E., Grosbois, V., Caron, A., Pople, D., Roche, B., & Donnelly, C. A. (2020). A systemic approach to assess the potential and risks of wildlife culling for infectious disease control. *Communications Biology*, *3*, 353. https://doi.org/10.1038/s42003-020-1032-z

Miller, M. W., Williams, E. S., Hobbs, N. T., & Wolfe, L. L. (2004). Environmental sources of prion transmission in mule deer. *Emerging Infectious Diseases*, *10*(6), 1003–1006. https://doi.org/10.3201/eid1006.040010

Mimura, M., Yahara, T., Faith, D. P., Vázquez-Domínguez, E., Colautti, R. I., Araki, H., Javadi, F., Núñez-Farfán, J., Mori, A. S., Zhou, S., Hollingsworth, P. M., Neaves, L. E., Fukano, Y., Smith, G. F., Sato, Y.-I., Tachida, H., & Hendry, A. P. (2016). Understanding and monitoring the consequences of human impacts on intraspecific variation. *Evolutionary Applications*, *10*(2), 121–139. https://doi.org/10.1111/eva.12436

Morand, S., & Walther, B. A. (2020). The accelerated infectious disease risk in the Anthropocene: more outbreaks and wider global spread. *BioRxiv*, 49866. https://doi.org/10.1101/2020.04.20.049866

Mysterud, A., & Rolandsen, C. M. (2018). A reindeer cull to prevent chronic wasting disease in Europe. *Nature Ecology and Evolution*, *2*, 1343–1345. https://doi.org/10.1038/s41559-018-0616-1

O'Rourke, K. I., Spraker, T. R., Hamburg, L. K., Besser, T. E., Brayton, K. A., & Knowles, D. P. (2004). Polymorphisms in the prion precursor functional gene but not the pseudogene are associated with susceptibility to chronic wasting disease in white-tailed deer. *The Journal of General Virology*, *85*(5), 1339–1346. https://doi.org/10.1099/vir.0.79785-0

Osterholm, M. T., Anderson, C. J., Zabel, M. D., Scheftel, J. M., Moore, K. A., & Appleby, B. S. (2019). Chronic wasting disease in cervids: implications for prion transmission to humans and other animal species. *mBio*, *10*(4), e01091–e1119. https://doi.org/10.1128/mBio.01091-19

Otsu, C., Iijima, H., & Nagaike, T. (2019). Plant community recovery from intense deer grazing depends on reduction of graminoids and the time after exclosure installation in a semi-natural grassland. *PeerJ*, *7*, e7833.

Palkovacs, E. P., Oppenheimer, A. J., Gladyshev, E., Toepfer, J. E., Amato, G., Chase, T., & Caccone, A. (2004). Genetic evaluation of a proposed introduction: the case of the greater prairie chicken and the extinct heath hen. *Molecular Ecology*, *13*(7), 1759–1769. https://doi.org/10.1111/j.1365-294X.2004.02181.x

Parchman, T. L., Gompert, Z., Mudge, J., Schilkey, F. D., Benkman, C. W., & Buerkle, C. A. (2012). Genome-wide association genetics of an adaptive trait in lodgepole pine. *Molecular Ecology*, *21*(12), 2991–3005. https://doi.org/10.1111/j.1365-294X.2012.05513.x

Patton, S. R., Russell, M. B., Windmuller-Campione, M. A., & Frelich, L. E. (2018). Quantifying impacts of white-tailed deer (*Odocoileus virginianus* Zimmerman) browse using forest inventory and socio-environmental datasets. *PLoS One*, *13*(8), e0201334. https://doi.org/10.1371/journal.pone.0201334

Peart, C. R., Tusso, S., Pophaly, S. D., Botero-Castro, F., Wu, C.-C., Aurioles-Gamboa, D., Baird, A. B., Bickham, J. W., Forcada, J., Galimberti, F., Gemmell, N. J., Hoffman, J. I., Kovacs, K. M., Kunnasranta, M., Lydersen, C., Nyman, T., de Oliveira, L. R., Orr, A. J., Sanvito, S., … Wolf, J. B. W. (2020). Determinants of genetic variation across eco-evolutionary scales in pinnipeds. *Nature Ecology & Evolution*, *4*, 1095–1104. https://doi.org/10.1038/s41559-020-1215-5

Pecl, G. T., Araújo, M. B., Bell, J. D., Blanchard, J., Bonebrake, T. C., Chen, I.-C., Clark, T. D., Colwell, R. K., Danielsen, F., Evengård, B., Falconi, L., Ferrier, S., Frusher, S., Garcia, R. A., Griffis, R. B., Hobday, A. J., Janion-Scheepers, C., Jarzyna, M. A., Jennings, S., … Williams, S. E. (2017). Biodiversity redistribution under climate change: impacts on ecosystems and human well-being. *Science*, *355*(6332), eaai9214. https://doi.org/10.1126/science.aai9214

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS One*, *7*(5), e37135. https://doi.org/10.1371/journal.pone.0037135

Phillips, P., Livieri, T. M., & Swanson, B. J. (2020). Genetic signature of disease epizootic and reintroduction history in an endangered carnivore. *Journal of Mammalogy*, *101*(3), 779–789. https://doi.org/10.1093/jmammal/gyaa043

Platt, A., Pivirotta, A., Knoblauch, J., & Hey, J. (2019). An estimator of first coalescent time reveals selection on young variants and large heterogeneity in rare allele ages among human populations. *PLoS Genetics*, *15*(8), e1008340. https://doi.org/10.1371/journal.pgen.1008340

Potapov, A., Merrill, E., Pybus, M., & Lewis, M. A. (2016). Chronic wasting disease: transmission mechanisms and the possibility of harvest management. *PLoS One*, *11*(3), e0151039. https://doi.org/10.1371/journal.pone.0151039

Price, S. J., Garner, T. W. J., Cunningham, A. A., Langton, T. E. S., & Nichols, R. A. (2016). Reconstructing the emergence of a lethal infectious disease of wildlife supports a key role for spread through translocations by humans. *Proceedings of the Royal Society B Biological Sciences*, *283*(1839), 20160952. https://doi.org/10.1098/rspb.2016.0952

Rivera, N. A., Brandt, A. L., Novakofski, J. E., & Mateus-Pinilla, N. E. (2019). Chronic wasting disease in cervids: prevalence, impact and management strategies. *Veterinary Medicine (Auckland, N.Z.)*, *10*, 123–139. https://doi.org/10.2147/VMRR.S197404

Rizzoli, A., Taliapietra, V., Cagnacci, F., Marini, G., Arnoldi, D., Rosso, F., & Rosà, R. (2019). Parasites and wildlife in a changing world: the vector-host-pathogen interaction as a learning case. *International Journal for Parasitology: Parasites and Wildlife*, *9*, 394–401. https://doi.org/10.1016/j.ijppaw.2019.05.011

Robinson, S. J., Samuel, M. D., Johnson, C. J., Adams, M., & McKenzie, D. I. (2012). Emerging prion disease drives host selection in a wildlife population. *Ecological Applications*, *22*(3), 1050–1059. https://doi.org/10.1890/11-0907.1

Rousselle, M., Simion, P., Tilak, M. K., Figuet, E., Nabholz, B., & Galtier, N. (2020). Is adaptation limited by mutation? A timescale-dependent effect of genetic diversity on the adaptive substitution rate in animals. *PLoS Genetics*, *16*(4), e1008668. https://doi.org/10.1371/journal.pgen.1008668

Samuel, M. D., Liao, W., Atkinson, C. T., & LaPointe, D. A. (2020). Facilitated adaptation for conservation – can gene editing save Hawaii's endangered birds from climate driven avian malaria? *Biological Conservation*, *241*, 108390. https://doi.org/10.1016/j.biocon.2019.108390

Schulte, L. A., Mladenoff, D. J., Crow, T. R., Merrick, L. C., & Cleland, D. T. (2007). Homogenization of northern U.S. Great Lakes forests due to land use. *Landscape Ecology*, *22*, 1089–1103. https://doi.org/10.1007/s10980-007-9095-5

Seabury, C. M., Oldeschulte, D. L., Bhattarai, E. K., Legare, D., Ferro, P. J., Metz, R. P., Johnson, C. D., Lockwood, M. A., & Nichols, T. A. (2020). Accurate genomic predictions for chronic wasting disease in U.S. white-tailed deer. *G3: Genes, Genomes, Genetics*, *10*(4), 1433–1441. https://doi.org/10.1534/g3.119.401002

Taylor, P. D., Crewe, T. L., Mackenzie, S. A., Lepage, D., Aubry, Y., Crysler, Z., Finney, G., Francis, C. M., Guglielmo, C. G., Hamilton, D. J., Holberton, R. L., Loring, P. H., Mitchell, G. W., Norris, D. R., Paquet, J., Ronconi, R. A., Smetzer, J. R., Smith, P. A., Welch, L. J., & Woodworth, B. K. (2017). The motus wildlife tracking system: a collaborative research network to enhance the understanding of wildlife movement. *Avian Conservation and Ecology*, *12*(1), 8. https://doi.org/10.5751/ACE-00953-120108

Thompson, T. Q., Bellinger, M. R., O'Rourke, S. M., Prince, D. J., Stevenson, A. E., Rodrigues, A. T., Sloat, M. R., Speller, C. F., Yang, D. Y., Butler, V. L., Banks, M. A., & Miller, M. R. (2019). Anthropogenic habitat alteration leads to rapid loss of adaptive variation and restoration potential in wild salmon populations. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(1), 177–186. https://doi.org/10.1073/pnas.1811559115

Walter, W. D., VerCauteren, K. C., Campa, H., Clark, W. R., Fischer, J. W., Hygnstrom, S. E., Mathews, N. E., Nielsen, C. K., Schauber, E. M., Van Deelen, T. R., & Winterstein, S. R. (2009). Regional assessment on influence of landscape configuration and connectivity on range size of white-tailed deer. *Landscape Ecology*, *24*, 1405–1420. https://doi.org/10.1007/s10980-009-9374-4

Weiskopf, S. R., Ledee, O., & Thompson, L. M. (2019). Climate change effects on deer and moose in the Midwest. *The Journal of Wildlife Management*, *83*(4), 769–781. https://doi.org/10.1002/jwmg.21649

White, K. S., Gregovich, D. P., & Levi, T. (2017). Projecting the future of an alpine ungulate under climate change scenarios. *Global Change Biology*, *24*(3), 1136–1149. https://doi.org/10.1111/gcb.13919

Wilson, G. A., Nakada, S. M., Bollinger, T. K., Pybus, M. J., Merrill, E. H., & Coltman, D. W. (2009). Polymorphisms at the PRNP gene influence susceptibility to chronic wasting disease in two species of deer (Odocoileus Spp.) in western Canada. *Journal of Toxicology and Environmental Health Part A*, *72*(17–18), 1025–1029. https://doi.org/10.1080/152873909030842

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.