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*)

Population and disease genomics of white-tailed deer.

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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 (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 cervids, 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 of cervids. We used high-throughput sequencing to assess neutral genomic variation, and variation in the PRNP gene 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 then 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 epidemic. Sustained surveillance of genomic and PRNP variation can be a useful tool for CWD-free regions where deer are managed for ecological and economic benefits.

Keywords

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

Introduction

Human-induced environmental change has caused widespread alterations to ecological and evolutionary processes (Harmon, Moran, & Ives, 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. Taylor et al., 2017; White, Gregovich, & Levi, 2017; Hashida et al., 2020). 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, Moen, Craft, & VanderWaal, 2019; Weiskopf, Ledee, & Thompson, 2019). In such instances, intraspecific genetic diversity and adaptive potential is crucial for long-term population viability (Kardos & Shafer, 2018).

The emergence, spread, and persistence of infectious diseases in previously allopatric populations is facilitated by climate change and other anthropogenic activity (Price et al., 2016; Aguirre, 2017; Morand & Walther, 2020). 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, Ledee, & Thompson, 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

64 multiple stressors simultaneously, or potentially face local extirpation (Fischer et al.,
65 2020).

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

80 A widespread threat to deer in North America is the highly infectious and fatal
81 neurodegenerative prion disease called chronic wasting disease (CWD). CWD is the
82 only prion disease known to infect captive and free ranging species of cervids (family
83 *Cervidae*) and has been reported in North and South America, Europe, and South
84 Korea (Haley et al., 2019). With virtually no barriers to transmission and a lengthy
85 infectious preclinical period, the local prevalence of CWD in North America has been

86 measured to be as high as 50% and 82% in wild and captive populations, respectively
87 (Miller et al., 2004; O'Rourke et al., 2004). Due to constraints on CWD surveillance it
88 is likely that the distribution and prevalence of CWD in wild populations are
89 underestimated (Escobar et al., 2020). It is clear the frequency and occurrence of CWD
90 has increased over time, in part driven by anthropogenic activities related to hunting
91 and wildlife farming (Osterholm et al., 2019).

92 Despite CWD being fatal there is inter-individual variation in susceptibility and
93 clinical progression. Susceptibility and clinical progression are associated with non-
94 synonymous and synonymous genetic variation in the functional prion protein gene
95 (PRNP; Güere et al., 2020; Chafin et al., 2020). Single nucleotide polymorphisms
96 (SNPs) at nucleotide (nt) 60, nt153, nt285, nt286, nt555, and nt676 in deer PRNP have
97 been associated with altered CWD susceptibility or pathogenic processes (Johnson et
98 al., 2006a; Wilson et al., 2009; Brandt et al., 2015; Brandt et al., 2018). The presence
99 of CWD appears to affect population PRNP allele frequencies over space and time due
100 to selection (Robinson et al., 2012); however, altered CWD susceptibility and
101 pathogenic processes are clearly polygenic traits (Seabury et al. 2020) and disease
102 spread is different in structured populations, which might require different wildlife
103 management practices (Chafin et al., 2020).The efficacy of selection in the face of a
104 novel pressure like CWD is dependent on the effective population size (N_e). Common
105 metrics to infer selection such as Tajima's *D*, specifically measure shifts in allele
106 frequencies across the site-frequency spectrum; however, the frequency and
107 proportion of rare alleles is sensitive to demographic processes (Messer, Ellner, &

108 Hairston Jr., 2016; Platt et al., 2019), and population genetics theory predicts an
109 excess of rare alleles in expanding populations (Gillespie, 2004).

110 Ontario, Canada reflects the northern leading edge of deer range in eastern
111 North America (Kennedy-Slaney, Bowman, Walpole, & Pond, 2018). The landscape of
112 Ontario is heterogenous and environmental clines exist around the Great Lakes
113 region. Ontario has not detected CWD in wild cervids, but CWD has been detected in
114 farmed and captive cervids from virtually every jurisdiction bordering Ontario, with
115 the province using a weighted surveillance approach to model CWD risk and
116 strategically use resources for surveillance. Accordingly, at the genome-level, we
117 predicted an excess frequency of rare neutral and PRNP variants across our study
118 region given Ontario's deer population is expanding (Kennedy-Slaney, Bowman,
119 Walpole, & Pond, 2018). Based on large-scale distribution changes and recent
120 population trends in deer (Baldwin, Desloges, & Band, 2000; Latch et al., 2009), we
121 predicted we would observe high neutral genomic diversity (N_e) and demographic
122 population expansion, indicating increased gene flow and decreased population
123 structure despite a heterogenous landscape. Since PRNP is not under selection by
124 CWD given the region is disease free, we predicted functional variation to resemble
125 regions most recently exposed to CWD (or still disease free). This is the first study to
126 characterize PRNP genetic variation and population genomic structure of wild deer,
127 while also determining the ancient and contemporary demographic and selection
128 processes driving patterns of diversity.

129

130 **Materials and Methods**

131 *Study area and sample collection*

132 We sampled white-tailed deer across Ontario, Canada (Figure 1). Between
133 2002-2018, retropharyngeal lymph nodes were opportunistically extracted from
134 hunter harvested deer across Ontario through the CWD surveillance program
135 managed by the Ontario Ministry of Natural Resources and Forestry (OMNRF).
136 Auxiliary data including year, sex, age-class, Mercator grid cell unit (GCU; 10x10 km),
137 and wildlife management unit (WMU) were also collected with deer samples. North
138 western and southern Ontario regions are geographically discontinuous for deer
139 (Figure 1), samples were therefore assigned to northern Ontario or southern Ontario
140 (Figure S1) for the purpose of analysis where sampling regions are compared.
141 Preliminary analysis of data did not warrant separating southeastern and
142 southwestern Ontario as per provincial management zones. Genomic DNA was
143 extracted from deer samples using a silica-based DNA extraction kit for tissue
144 following manufacturers protocol (Qiagen, Cat. No. 69506) and stored at -20°C. DNA
145 quality was assessed by a spectrometer (NanoDrop 2000, Thermo Scientific) and by
146 2% agarose GelRed gel electrophoresis.

147 *Library preparation for PRNP genetic analysis*

148 A 771 base-pair (bp) region of the deer prion protein precursor (PRNP) gene
149 was targeted and amplified using four degenerate primers (Table S1). Four replicate
150 PCRs generated a 460 base pair Fragment 1 and a 580 base pair Fragment 2 (Table
151 S2). In a 2:1 ratio of Fragment 1 to Fragment 2, respectively, amplified DNA was added

152 for each individual and then indexed with standard Illumina multiplexing indices.
153 Negative controls of UltraPure distilled water (Invitrogen, 1897011) were used for
154 each 96-well plate. The library was purified of artifacts following manufacturers
155 protocol for AMPure XP beads (Beckman Coulter, A63880) and validated with a
156 TapeStation D1000 kit (Agilent, 5067-5582). The library was sequenced on an
157 Illumina MiSeq platform at the University of Guelph Advanced Analysis Centre to
158 generate 300 base pair (bp) pair-end reads for each sample.

159 *Library preparation for RADseq genomic analysis*

160 Restriction-site associated DNA sequencing (RADseq) libraries were
161 generated using an adapted protocol from Parchman et al. (2012) and Peterson et al.
162 (2012) with Sbfl-HF and Msel restriction enzymes. Samples were incubated, digested
163 overnight, and heat-inactivated in 96-well plates (Table S3). Negative controls of
164 UltraPure distilled water (Invitrogen, 1897011) were used for each 96-well plate.
165 Restriction digested DNA was combined with 7 ul of ligation mixture and 3 ul of one
166 of the 24 available Sbfl adapters (1.0 uM). Adapters were ligated at 16°C for 3 hours.
167 DNA fragments were purified of artifacts following manufacturers protocol for
168 AMPure XP beads (Beckman Coulter, A63880). Adapter-ligated fragments were
169 amplified in four separate 10 ul reactions that incorporated barcodes. Reaction
170 conditions and primers are shown for ligation mixture and PCR in Table S4 and Table
171 S5, respectively. Samples were pooled and purification was performed following
172 manufacturers protocol for QIAquick PCR Purification kit (Qiagen, 28106) for a final
173 elution to 42 ul. Size selection between 450 bp to 700 bp was performed on 80 ul

174 replicates of purified library and gel purification was performed following
175 manufacturers protocol for QIAquick Gel Extraction kit (Qiagen, 28706) for a final
176 elution to 60 ul. The purified final library was validated with a TapeStation D1000 kit
177 (Agilent, 5067-5582). The libraries were sequenced at The Centre for Applied
178 Genomics (TCAG) in The Hospital for Sick Children (SickKids) on an Illumina HiSeq
179 2500 to produce 2x126 base pair paired end reads.

180 *Bioinformatic pipeline and data analysis I – PRNP gene*

181 The quality of reads was assessed using FastQC (Babraham Institute; v0.11.8).
182 Samples were excluded if at least one file in the pair-end files for a sample was less
183 than 1kB in size or failed to pass quality standards. A novel command line-based
184 pipeline was developed to assemble and genotype PRNP (accessible at
185 https://gitlab.com/WiDGeT_TrentU). Our workflow integrated Pullseq v1.0.2, BWA
186 v0.7.17, SAMtools; v1.9, BCFtools v1.9, and VCFtools v0.1.16-15. Briefly, for each
187 sample, the pipeline extracted relevant reads based on the presence of primer
188 sequence, mapped the extracted reads to a 771 bp PRNP gene reference sequence. We
189 generated a consensus sequence and called single nucleotide polymorphisms (SNPs).
190 SNP calls were limited to positions where there was a minimum read depth of 30 and
191 mapping quality score of at least 30. Sanger sequencing of a subset of samples and
192 their a priori called variants were used to validate the bioinformatic pipeline.

193 The presence of asparagine (N) at aa138 (nt413A) indicates amplification of
194 the pseudogene (Brandt et al., 2015); we therefore filtered out all sequences with this
195 site. SNPs with a total frequency of occurrence of 1% or less were excluded from the

196 analysis. A two-sided Fisher's Exact Test was conducted on minor allele counts from
197 either northern or southern sampling regions at four well studied positions in the
198 deer PRNP gene associated with CWD: nucleotide (nt) 60, nt285, nt286, and nt676.
199 Synonymous and non-synonymous sites were identified using MEGA X v10.0.5.
200 Haplotypes were estimated from unphased sequences with PHASE v2.1.1 using a
201 Markov chain Monte Carlo (MCMC) sampling approach with a minimum of 100,000
202 steps, with a discarded burn-in of 10,000, and samples were drawn every 100 MCMC
203 steps. Five repetitions were performed to verify consistent frequencies of haplotype
204 assignment (Brandt et al., 2018). Haplotypes with a frequency of less than 1% were
205 removed. The genotype, frequencies, and estimated standard deviations of the
206 remaining haplotypes were analyzed as a 2x2 contingency table by sampling region.
207 *Bioinformatic pipeline and data analysis II – RADseq*
208 Fastq files were demultiplexed using process_radtags within the Stacks v2.3
209 module. Parameters within process_radtags included the removal of any read with an
210 uncalled base and the discarding of reads with low quality scores. The demultiplexed
211 sample files were aligned against the deer genome (Genome Accession
212 JAAVWD000000000) using BWA with samtools used to sort, merge and compress
213 BAM files. The referenced-based approaches on gstacks and populations program
214 within STACKs produced a variant call format (VCF) file with the restrictions that the
215 minimum percentage of individuals in a population required to process a locus for
216 that population was 90%. The VCF was filtered using VCFtools to only include reads
217 with a minimum read depth of 20. Population statistics, including F_{IS}, observed and

218 estimated homozygosity, and nucleotide diversity were calculated using the
219 *populations* module. To analyze sources of variation, we generated a principal
220 component analysis (PCA) using the R v3.6.1 package adegenet v2.1.3. A linear
221 regression was run on principal component (PC) 1 and PC2 scores against latitude
222 and longitude. We estimated F_{ST} between north and south using StAMPP v1.6.1.
223 Population structure was detected using successive K-means clustering and a
224 discriminant analysis of principal components (DAPC) available in adegenet (Jombart,
225 Devillard, & Balloux, 2010).

226 *Demographic Analysis and Estimate of Effective Population Size*

227 The final VCF was converted into 1D site frequency spectrum (SFS) for all of
228 Ontario and northern vs and southern designations, respectively, using vcf2dadi.py
229 with projections for the SFS estimated in easySFS. We applied a diffusion-based
230 approach to demographic inference through the Diffusion Approximation for
231 Demographic Inference (δaδi) tool by Gutenkunst et al., (2009). Nine 1D models were
232 assessed for Ontario as a single population. The optimum model was selected as the
233 lowest optimized log-likelihood of all successfully run models. δaδi was also used to
234 estimate the following summary statistics for the province: Watterson Theta (θ),
235 Tajima's *D* and the number of segregating sites. Using the mutation rate (μ) per site
236 per generation of a closely related species (*Rangifer tarandus* from Chen et al., 2019),
237 total number of sites (L), and the parameters estimated in the optimum model
238 selected from δaδi, we estimated the ancestral effective population size as $N_a = \theta /$
239 $4\mu L$.

240 *Estimation of selection on PRNP and allele frequency projections for a naïve population*

241 We estimated the selection coefficient (s) at two sites (nt285 and nt286) using
242 the approach of Thompson et al. (2019) which accounts for number of generations
243 and different expression modes (i.e. recessive, dominant, codominant). Strength of
244 selection against the less resistant phenotype [i.e., the homozygous common allele
245 (s_{AA})] can be estimated by calculating values of s_{AA} that explained the estimated
246 change in allele frequencies between positive and negative animals from the same
247 region. Here we used starting (negative CWD) allele frequencies from Wilson et. al.
248 (2009) and Kelly et al. (2009) and estimated s over n generations the equation:

249 $$p' = (1-s_{AA})p^2 + (1-s_{AB})p(1-p) / (1-s_{AA})p^2 + (1-s_{AB})2p(1-p) + (1-s_{BB})(1-p)^2)$$

250 taken from Charlesworth and Charlesworth (2010). To account for uncertainty in
251 time and allele frequency estimates we ran 100 iterations with n ranging from 2-25
252 generations (~4-50 deer years) and positive and negative allele frequencies (±1%)
253 for those reported from both Wilson et. al. (2009) and Kelly et al. (2009). Then using
254 our estimated allele frequencies for Ontario, we projected each allele frequencies into
255 the next 25 generations using our estimated selection coefficients, and the estimated
256 s coefficients of 0.0103 and 0.074 from Robinson et al. (2012). Calculations were
257 conducted under three relative fitness scenarios as per Thompson et al. (2019).
258

259 **Results**

260 *PRNP Genetic analysis*

261 A total of 631 Ontario deer samples were included in the PRNP genetic analysis
262 (Figure 1). Nineteen SNPs were detected after filtering (Table 1), with 8 being non-
263 synonymous substitutions. Six of the detected variants in the PRNP gene have been
264 linked to CWD susceptibility or clinical progression, these include nt60, nt153, nt285,
265 nt286, nt555, and nt676. A two-sided Fisher's Exact test on the major and minor allele
266 counts at the four important, arguably the most studied, CWD-linked loci (nt60, nt285,
267 nt286, and nt676) conducted between Northern and Southern Ontario indicated that
268 there was only a difference in frequency ($p < 0.05$) at nt676 (Table S6).

269 There were 102 unique haplotypes with a count of at least one, with 12
270 haplotypes having a frequency greater than 1% (Figure 2; Table S7). The two most
271 common haplotypes, Haplotype 3 (f=0.23) and Haplotype 1 (f=0.12) did not include
272 any non-synonymous substitutions. Haplotype A (f=0.30) and Haplotype B (f=0.25)
273 reported by Brandt et al (2015, 2018) from northern Illinois were also detected as
274 Haplotype 16 (f=0.09) and Haplotype 7 (f=0.05), respectively. The same Haplotype A
275 (f=0.15) and Haplotype B (f=0.23) were reported by Chafin et al., 2020 in deer from
276 Arkansas, USA.

277 *RADseq Genomic analysis*

278 A total of 235 Ontario deer samples were sequenced in ddRADseq libraries.
279 Following quality control and quality assessment, including FastQC and line counts of
280 demultiplexed files, 190 samples remained for downstream analysis (Figure 1).

281 Estimated population diversity statistics are summarized in Table 2. The PCA clearly
282 separated northern and southern Ontario along PC1 (Figure 3). A linear regression
283 revealed that PC1 was strongly associated with longitude ($\beta = -0.79$; adjusted R^2 =
284 0.77; p-value < 0.01). However, population structure between northern and southern
285 Ontario was weak (F_{ST}=0.02). A BIC based on the K-cluster analysis also indicated that
286 the most optimum number of clusters was 1.

287 The optimum 1D demographic model for Ontario was the BOTTLEGROWTH
288 model, which models an instantaneous size change followed by exponential change
289 (Table S8; Figure 4). From the 1D site frequency spectrum, the mean Tajima's *D* was
290 estimated to be -2.126 consistent with a population expansion after a bottleneck.
291 Using the calculated θ, we estimated N_a to be ~20,000: this would place the timing of
292 the population change (Tc) measured in $2N_a$ generations around the onset of the last
293 glacial maximum (Figure 4).

294 *PRNP selection and projection*

295 We estimated the selection coefficients to be 0.08 (±0.06) and 0.11 (±0.07) for
296 nt285 and nt286 under a dominance model. All simulated projections with our *s*
297 values and the upper reported value of Robinson et al (2012) showed a rapid shift in
298 allele frequencies (Figure 5); the majority of simulated trajectories did not overlap
299 with the low *s* coefficient of 0.01 previously reported by Robinson et al (2012).

300 **Discussion**

301 White-tailed deer in North America are intensively managed for hunter
302 harvest and are expanding their range northward due to climate change. The

303 frequency and occurrence of CWD infection in captive and free-ranging deer is
304 likewise increasing (Osterholm et al., 2019; Rivera, Brandt, Novakofski, & Mateus-
305 Pinilla, 2019), but transmission and spread in free-ranging populations are still poorly
306 understood (Potapov, Merrill, Pybus, & Lewis, 2016). We observed that the frequency
307 of PRNP alleles in our naïve population differed from areas currently infected with
308 CWD or where CWD is endemic, including western Canada (Table S8; Kelly et al.,
309 2008; Wilson et al., 2009; Brandt et al., 2015; Brandt et al., 2018; Chafin et al., 2020).
310 The emergence, transmission, and persistence of highly infectious diseases in
311 healthy populations are often facilitated by climate change and exacerbated in areas
312 with intense anthropogenic activity (McKnight et al., 2017; Rizzoli et al., 2019). The
313 introduction and re-introduction of infectious diseases often results in rapid local
314 population declines, reducing species' adaptive potential and generating substantial
315 economic losses (Belant & Deese, 2010; Escobar, Moen, Craft, & VanderWaal, 2019).
316 In Ontario deer, however, we observed an excess of rare alleles with no evidence of
317 strong population structure, including in southern Ontario where an environmental
318 cline exists around lake systems. An excess of rare alleles and high N_e and absent
319 population structure might provide the means for effective adaptation to selective
320 pressures, including climate change, infectious disease, and human activity.
321 *Linking Population Demographics to Functional genes*
322 We assessed contemporary and historical gene flow by examining neutral
323 genomic variation across tens of thousands of loci from deer across the province on
324 Ontario. The data were consistent with a large expanding population with a high level

325 of genomic diversity and an excess of rare alleles. These genomic patterns are also
326 consistent with population simulations of responses to climate change (Kennedy-
327 Slaney, Bowman, Walpole, & Pond, 2018), and offers some clues as to the potential
328 adaptive response were CWD to arrive. Specifically, two important features of the
329 genomic data are noteworthy: the high number of a rare alleles and the limited
330 population structure across Ontario.

331 Demographic processes and life history strategies influence the proportion of
332 rare alleles, which are important to the adaptive process but are sensitive to recent
333 evolutionary processes (Peart et al. 2020). Evidence suggests the accumulation of rare
334 alleles is independent of taxa, but adaptation appears limited in low-diversity taxa
335 (e.g., primates; Rousselle et al., 2020). The deer genomic data evaluated here are
336 consistent with high adaptive potential; specifically, we calculated an N_a to be
337 ~20,000, with the expansion estimated to be 100X suggestive of a large species-wide
338 N_e (derived either from π in Table 2 or the demographic model in Figure 4).

339 Studies of populations infected with CWD often demonstrate a lack of allelic
340 diversity at the PRNP gene, which is thought to be due to CWD-driven selection
341 positively selecting for functionally relevant alleles (Haley et al., 2019). We found a
342 high frequency of rare PRNP alleles in our naïve population which differs from areas
343 currently infected or where CWD is endemic, including western Canada (Johnson et
344 al., 2006a; Kelly et al., 2008; Wilson et al., 2009; Brandt et al., 2015; Brandt et al.,
345 2018). Furthermore, infectious diseases can cause population fragmentation (i.e.,
346 structure), demographic changes, and genetic isolation (McKnight et al., 2017). A pre-

347 infection presence of a large proportion of rare alleles in both functional and neutral
348 genes, as observed in Ontario, supports surveillance data showing no CWD, which
349 might bode well for a long-term adaptive response to CWD infection and other
350 stressors. Importantly, even low selection coefficients should alter allele frequencies
351 in a detectable manner (Figure 5). However, it is not clear how this standing genetic
352 variation compares to infected populations prior to the arrival of CWD, creating some
353 uncertainty in the potential adaptive response, recognizing that loci outside PRNP are
354 also involved (Seabury et al. 2020).

355 We observed no evidence of strong population structure despite the
356 heterogeneous landscape observed in Ontario, but there is a clear latitudinal cline in
357 allele frequencies. This suggests random mating is largely occurring a regional levels
358 in spite of substantial environmental changes including intense agricultural practices,
359 substantial urbanization of the landscape, and climate change (Schulte et al., 2007;
360 Walter et al., 2009; Patton, Russell, Windmuller-Campione, & Frelich, 2018). We might
361 therefore expect that the spread of advantageous rare alleles under selection may not
362 be limited in the province. Conversely, we might also expect the homogenous
363 population to facilitate the spread of CWD if anthropogenic activity were to introduce
364 the disease into the focal population (Escobar et al., 2020). The movement of infected
365 wildlife might also pose a potential risk to human health at the wildlife-human-
366 livestock interface as CWD can cross species barriers (Igel-Egalon et al., 2020).
367 Sustained monitoring across CWD-free regions where deer are managed for
368 sustainability or where food security is threatened should continue, but consider

369 population-level responses to climate change (i.e. Kennedy-Slaney, Bowman, Walpole,
370 & Pond, 2018) while integrating genetic information beyond PRNP allele frequencies.

371 *Conclusion*

372 We gauged the adaptive potential of CWD naïve deer in Ontario, Canada by
373 assessing functional and neutral genetic diversity. The genome-wide data were
374 consistent with a large expanding population with a high neutral genomic diversity,
375 no population structure, and an excess of rare alleles, which is also consistent with
376 non-genetic population and climate models (Kennedy-Slaney, Bowman, Walpole, &
377 Pond, 2018). We suggest these patterns might favour a novel adaptive response to the
378 arrival of CWD. Voluntary hunter-harvest based surveillance for CWD will likely be
379 able to detect the introduction of CWD in a naïve population; however, we expect that
380 there will be a lag in detection when prevalence is low since low densities likely limit
381 transmission (Gagnier, Laurion, & DeNicola, 2020). Unfortunately, a lag in detection
382 will likely permit the establishment of CWD and, over time, eradication becomes
383 nearly impossible and costs are high (Mysterud & Rolandsen, 2018). Sustained
384 temporal monitoring of variation in PRNP across CWD-free regions could be a
385 detection tool as our simulations suggest detectable shifts should occur with the
386 arrival of the disease. By combining demographic patterns and genotypes with
387 current risk models, managers could improve risk-based detection efforts and
388 facilitate a more effective resource deployment plan as the disease alters the
389 population.

390

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Data Accessibility

DNA sequences: SRA Accessions PRJNA565222 and SUB8436966

PRNP and RADseq analysis pipelines: https://gitlab.com/WiDGeT_TrentU/

414 **Tables**

415 **Table 1**. Allele and amino acids frequencies for reference/alternate alleles at Ontario
416 white-tailed deer (n=631) prion protein gene.

Locus	Allele	Codon	Amino acid	Major frequency	Minor frequency
60	C/T	20	D/-	0.9794	0.0206
136†	A/G	46	S/G	0.9873	0.0127
153	C/T	51	R/-	0.8605	0.1395
168†	A/G	56	G/-	0.9635	0.0365
174†	T/G	58	G/-	0.9842	0.0158
177†	C/G	59	G/-	0.9873	0.0127
178†	T/G	60	W/G	0.9160	0.0840
192†	T/G	64	H/Q	0.8653	0.1347
195†	A/G,T	65	G/-	0.7147	0.2853
198†	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†	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†	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

417 † indicates novel positions.

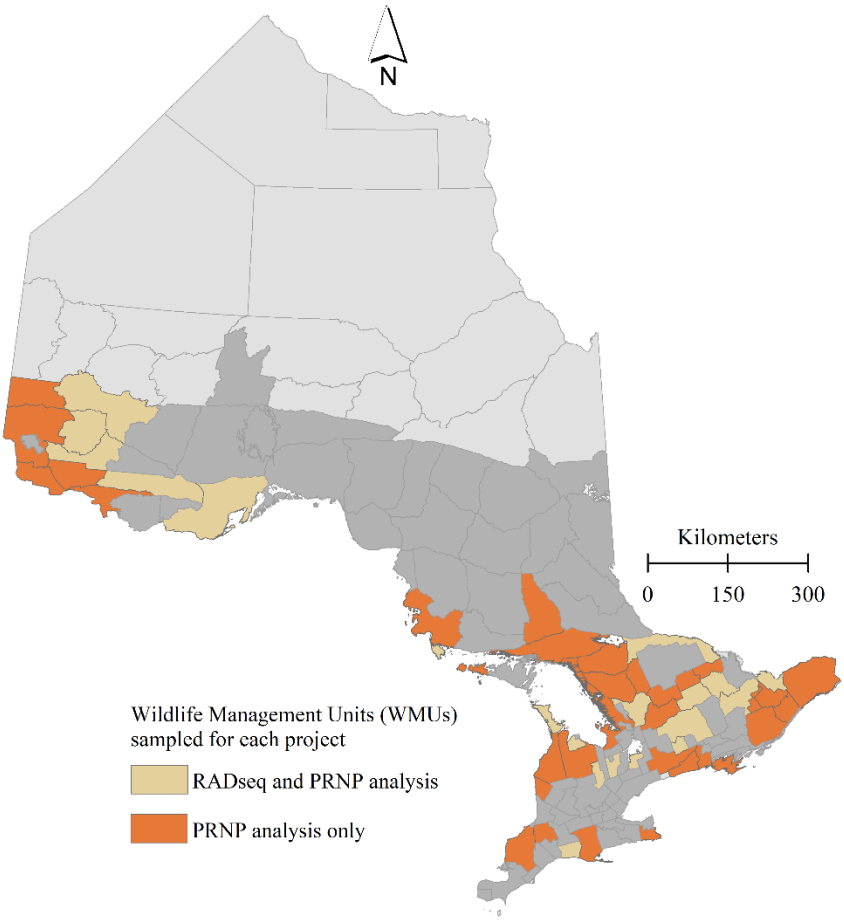
418

419 **Table 2**. Genome-wide population summary statistics including breakdown of sites,
420 number of individuals, nucleotide diversity estimate (π), individual genetic variance
421 (I) relative to the subpopulation genetic variance (F_{IS}), and the observed
422 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	$5.0x10^{-4}$	$6.4x10^{-4}$	$5.7x10^{-4}$
Observed heterozygosity	$4.7x10^{-4}$	$6.1x10^{-4}$	$5.5x10^{-4}$
F_{IS}	$5.7x10^{-4}$	$3.9x10^{-4}$	$4.2x10^{-4}$

423

424 **Figures**



425

426 **Figure 1**. Distribution of free-ranging white-tailed deer samples obtained between
427 2003-2018 by the Ontario Ministry of Natural Resources and Forestry (OMNRF) that
428 were used for the reduced representation genome analysis (n=190; cream) and the
429 prion protein genetic analysis (n=631; orange *and* cream). The natural distribution of
430 free-ranging white-tailed deer is shown for Ontario with a darker shade of gray.

431

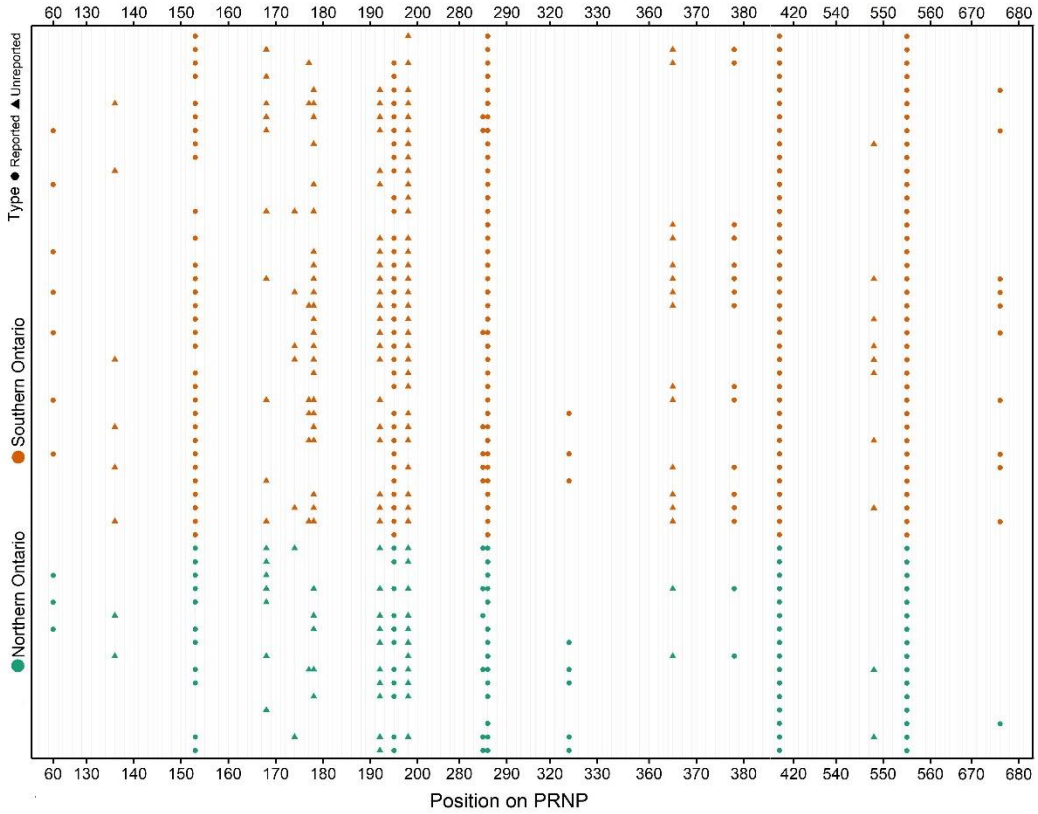


Figure 2. A 771 bp region of the white-tailed deer prion protein gene was analyzed from free-ranging white-tailed deer in Ontario, Canada (n=631). The overlayed genotypes across 19 variable loci were organized by broad management in Ontario and are shown. Circles indicate loci previously described as variable in the white-tailed deer prion protein gene. Triangles indicate novel variable loci in the variable loci in the white-tailed deer prion protein gene.

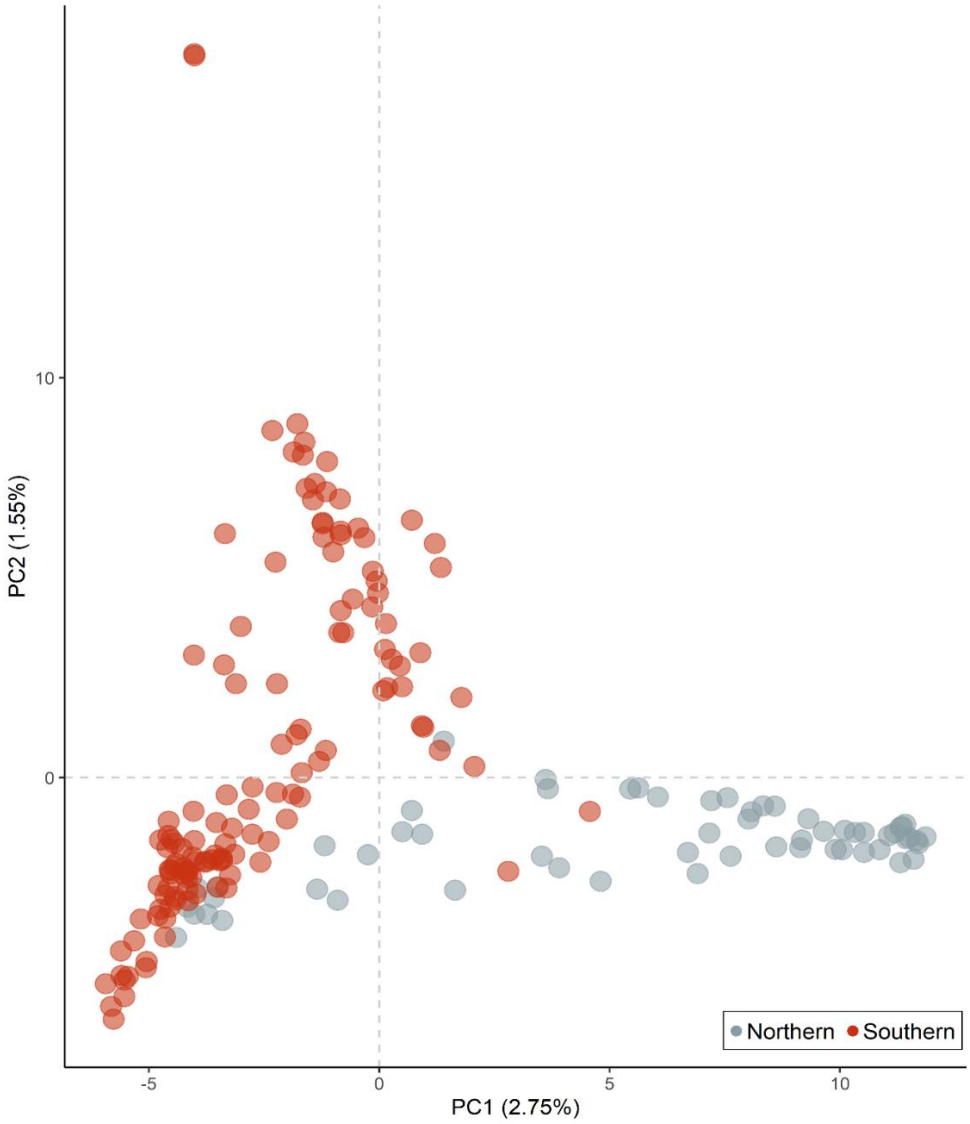
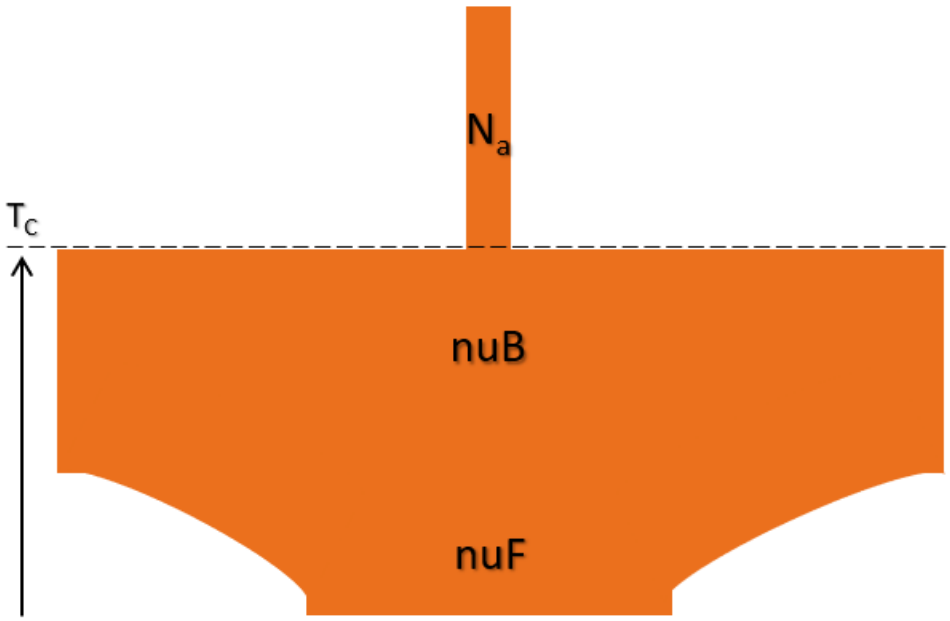


Figure 3. A plot of PC1 and PC2 from the principal component analysis (PCA) on the reduced representation white-tailed deer genome. PC1 and PC2 were able to explain a total of 4.3% of the genomic variation observed. Gray circles represent scores from samples in northern Ontario. Orange circles represent scores from samples in southern Ontario.



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

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 (N_a), 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 (T_C; in units of $2*N_a$ generations). Included are the optimized log-likelihood (LL) and bootstrap uncertainties (BU).

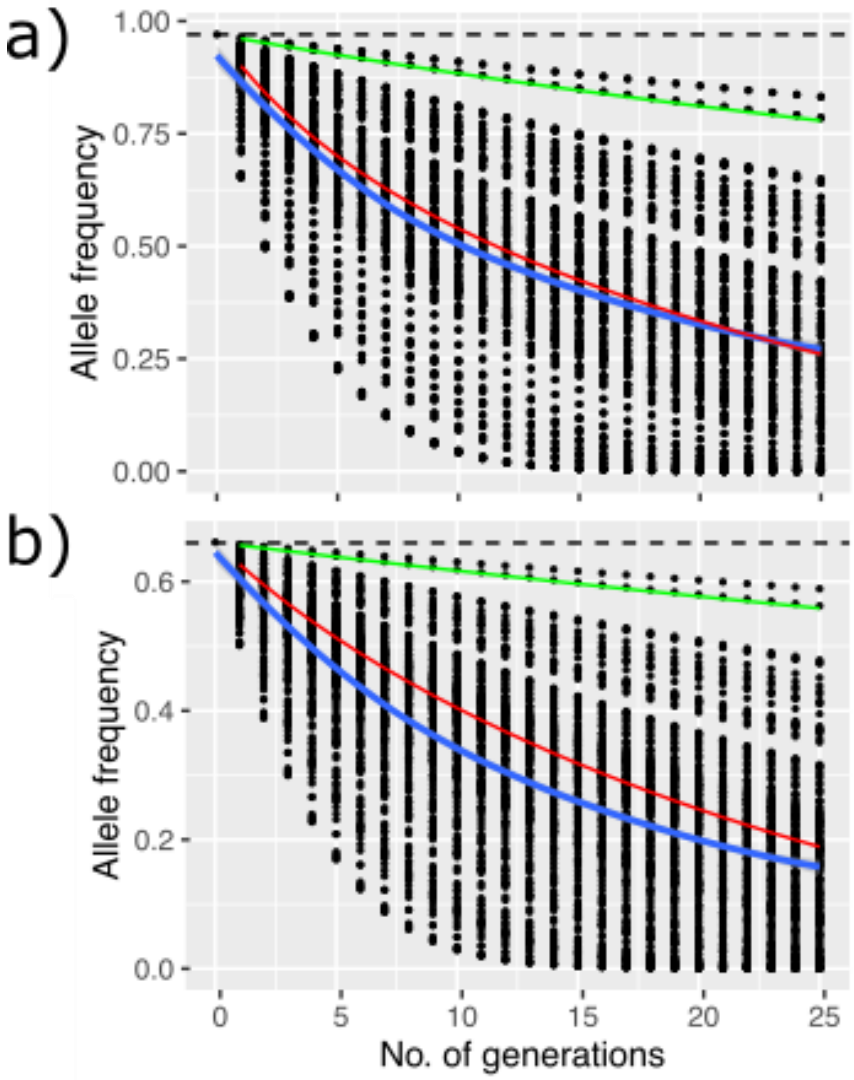


Figure 5. Simulated allele frequency projections for nucleotide positions 285 (a) and 286 (b) in the PRNP gene under selection. Green and red lines are the selection coefficients provided by Robinson et al (2012); while the black dots are those from our simulations, with blue line representing the mean selection coefficient.

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740 **Supplementary Materials**

741 **Table S1**. Four degenerate primers used to amplify the functional white-tailed deer prion
742 protein gene region as two overlapping fragments are shown. Fragment 1 is 460 base-pairs
743 in length and Fragment 2 is 590 base-pairs in length.

Primer	Fragment 1	Fragment 2
Forward (5'-3')	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACRTGGGCATATGATGCTGAYACC	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGGAGGCTGGGGTCAAGG
Reverse (5'-3')	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGYTGCCAAAATGTATAAGAGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACTACAGGGCTGCAGGTAGAYACT

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758 **Table S2**. Thermal cycling conditions for PRNP PCR amplification for Fragment 1 and
759 Fragment

Fragment 1	Fragment 2
98°C for 2 min	
10 cycles of:	
98°C for 15 sec	98°C for 15 sec
60-55°C for 30 sec[1]	65-60°C for 30 sec[1]
72°C for 45 sec	72°C for 45 sec
98°C for 15 sec	
20 cycles of:	
98°C for 15 sec	98°C for 15 sec
55°C for 30 sec	60°C for 30 sec
72°C for 45 sec[2]	72°C for 45 sec[2]
72°C for 7 min	

1 – decrease by 0.5° per cycle; 2 – increasing by 5 sec per cycle

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767 **Table S3**. Mixture for double restriction enzyme digestion. Samples and reaction mixture
768 were incubated at 37°C for 3 hours and then 25°C overnight on a thermal cycler with a heated
769 lid. Samples and reaction mixture were head-inactivated at 85°C for 30 minutes.

Reagent	1 X	Final concentration
10x Cutsmart	2.00 ul	1 X
H_2O	11.85 ul	
MseI (10 U/ µl)	0.10 ul	1 U
SbfI-HF (20 U/ µl)	0.05 ul	1 U
Subtotal	14.00 ul	
DNA	x ul	~ 500 ng
H_2O	6.00 – x ul	
Subtotal	6.00 ul	
Final Volume	20.00 ul	

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771 **Table S4**. Mixture for adapter ligation. The digested fragments were combined with 7 ul of
772 the adapter ligation mixture and 3 ul of a unique barcoded SbfI adapter (1.0 uM). The ligation
773 on ~ 30 ul reaction mixture was performed in 16°C for 3 hours

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Reagent	1 X	Final concentration
10x CutSmart	1.00	1 X
100 mM ATP	0.30	1 mM
H_2O	2.45	
MseI Y adapter (10 uM)	3.00	1 µM
T4 DNA ligase (400 U/µl)	0.25	100 U
Subtotal	7.00 ul	

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776 **Table S5**. Illumina PCR mixture. The purified restriction-ligation DNA (3 ul) was combined
777 with 7 ul of PCR mixture and a PCR was performed on 10 ul. Four replicated per sample was
778 performed. The thermal cycler profile for this PCR was 98°C for 30 seconds; 20 cycles of 98°C
779 for 20 seconds, 60°C for 30 seconds, 72°C for 45 seconds; and a final extension at 72°C for 5
780 minutes.

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Reagent	1 X	Final concentration
H_2O	1.33	
KAPA HiFi ReadyMix	5.00	2 X
PCR primer mix (5 µM each)	0.67	0.5 µM
Subtotal	7.00 ul	

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784 **Table S6**. The major/minor allele counts for four nucleotide (nt) positions of variation in the
785 white-tailed deer prion protein gene that are linked to reduced susceptibility or reduced
786 clinical progression of chronic wasting disease for Northern and Southern Ontario are shown.
787 A two-sided Fisher's Exact test was conducted on the major and minor allele counts at the
788 four chronic wasting disease-linked nucleotide positions. A p-value less than 0.05 was
789 considered significant and indicated that there were significant differences between the
790 groups.

Location	nt60	nt285	nt286	nt676
Northern	186/3	182/7	132/57	187/2
Southern	432/10	430/12	285/157	420/22
Southeastern	306/8	308/6	196/118	300/14
Southwestern	126/2	122/6	89/39	120/8
p-value	0.764	0.611	0.200	0.021

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Table S7. Haplotypes were estimated with PHASE v2.1.1 set to the same parameters in Brandt et al., 2018: Markov chain Monte Carlo (MCMC) samples were taken from a minimum of 100,000 steps, with a discarded burn-in of 10,000; samples were drawn every 100 MCMC steps. Five repetitions were performed, and haplotype frequencies compared to verify consistent assignment. Included are estimates of population haplotypes with frequencies of greater than 1% (count=1262, number haplotypes = 151) and associated estimated standard deviations (S.E.; square root of the variance of the posterior distribution) at 19 variable positions, with 0 representing non-variants and 1 representing variants.

ID	f	S.E.	Codon	153	195	198	286	365	378	417	555
3	0.228	0.006	-	0	0	0	0	0	0	1	0
1	0.122	0.005	-/-	0	0	0	0	0	0	1	1
9	0.104	0.001	Ref	0	0	0	0	0	0	0	0
16	0.087	0.005	96S/-/-	0	0	0	1	0	0	1	1
7	0.050	0.001	-	0	0	0	0	0	0	0	1
252	0.041	0.003	-/-	1	0	0	0	0	0	1	0
18	0.033	0.004	96S/-	0	0	0	1	0	0	1	0
54	0.022	0.003	-/-/-	0	1	1	0	0	0	1	0
259	0.012	0.002	-/122V/-/-	1	0	0	0	1	1	1	0
27	0.012	0.002	-/-/-	0	0	1	0	0	0	1	1
28	0.011	0.003	-/96S/-/-	0	0	1	0	0	0	1	0
48	0.010	0.002	-/96S/-/-	0	1	0	1	0	0	1	1

Table S8. Optimized log-likelihood (LL) and bootstrap uncertainties (BU) obtained from 1D demographic models on Ontario white-tailed deer as a single population. Model specifics and parameters are outlined. The most optimal model for 1D is shown in bold. Modified 1D demographic models are indicated with an asterisk. All time estimates are reported in units of 2*Na generations. Migration rate (m) is reported in units of 2*Na*m.

Model Name	LL	Parameter	Estimate	BU
SNM	-25746	n/a	n/a	41.3 $1.11x10^{-15}$ $7.77x10^{-16}$
TWO_EPOCH	-3015	nu	573.38	3.97 40.69
		T	107.90	7.62
GROWTH	-8795	nu	21.57	217.02 8.93
		T	6.58	3.79
BOTTLEGROWTH	-2034	nuB	548.48	35.37
		nuF	139.97	30.83
		T	26.10	85.78
BOTTLEPOP*	-8495	nu	54.48	287.41
		T	20.17	78.28 46.48
TWOPOPCHANGES	-3129	nuB	$4.19x10^{-5}$	$5.16x10^{4}$
		nuF	0.32	
		TB	$5.14x10^{-3}$	$2.06x10^{-5}$

		TF	$6.04x10^{-2}$	0.11
GROWTHPLUSBOTTLE	-25631	nuB	8.47	$1.43x10^{5}$
		nuF	0.14	
		TB	0.92	2.74
		TF	4.28	$2.39x10^{-2}$
BOTTPLEPLUSGROWTH	-3544	nuB	397.36	8.75
		nuF	473.75	93.69
		T	97.40	169.23
THREE_EPOCH	-3274	nuB	$1.35x10^{-4}$	$1.99x10^{5}$
		nuF	$1.31x10^{-2}$	$1.46x10^{-5}$
		TB	$3.48x10^{-3}$	$2.64x10^{-3}$
		TF	$2.47x10^{-3}$	

822 **Table S9**. Nucleotide variations in free-ranging white-tailed deer prion protein gene that are
823 associated with chronic wasting disease are either protective (1), increase susceptibility (2),
824 or are neutral. The major and minor allele frequencies for each site across a 771 bp region of
825 the prion protein gene in white-tailed deer are reported for different regions, in descending
826 order. The year CWD was found in free-ranging cervids is reported for each location. The data
827 are from free-ranging white-tailed deer samples collected in: Alberta, Canada (AB); Colorado,
828 USA (COL); Illinois, USA (IL); Ontario, Canada (ON); Nebraska, USA (NE); Saskatchewan,
829 Canada (SK); Wisconsin, USA (WI); and Wyoming, USA (WY).

Site	Role	Major	Minor	Region	CWD+	Citation
C60T	1	0.98	0.02	ON	n/a	Haworth et al., 2020
		0.98	0.02	WY	1985	Heaton et al., 2003
		0.94	0.06	AB, SK	2002,1996	Wilson et al., 2009
		0.92	0.08	IL	2002	Kelly et al., 2008
C153T	2	0.96	0.04	WY	1985	Heaton et al., 2003
		0.94	0.06	AB, SK	2002,1996	Wilson et al., 2009
		0.89	0.11	IL	2002	Kelly et al., 2008
		0.86	0.14	ON	n/a	Haworth et al., 2020
A285C	1	1.00	0.00	WI	2002	Johnson et al., 2006
		0.99	0.01	AB, SK	2002,1996	Wilson et al., 2009
		0.98	0.02	NE	1999	Vázquez-Miranda & Zink, 2020
		0.97	0.03	ON	n/a	Haworth et al., 2020
		0.94	0.06	IL	2002	Kelly et al., 2008
G286A	1	0.88	0.12	COL	1967	O'Rourke et al., 1998 (unpublished)
		0.86	0.14	IL	2002	Kelly et al., 2008
		0.83	0.17	NE	1999	Vázquez-Miranda & Zink, 2020
		0.81	0.19	WI	2002	Johnson et al., 2006

		0.66	0.34	ON	n/a	Haworth et al., 2020
A324G	1	0.98	0.02	ON	n/a	Haworth et al., 2020
		0.98	0.02	NE	1999	Vázquez-Miranda & Zink, 2020
		0.96	0.04	WY	1985	Heaton et al., 2003
		0.96	0.04	AB, SK	2002,1996	Wilson et al., 2009
		0.94	0.06	WY	1985	Heaton et al., 2003
G417A	3	0.99	0.01	AB, SK	2002,1996	Wilson et al., 2009
		0.79	0.21	COL	1967	O'Rourke et al., 1998
		0.17	0.83	ON	n/a	Haworth et al., 2020
C555T	1	0.89	0.11	n/a	n/a	Raymond et al., 2000
		0.65	0.35	AB, SK	2002,1996	Wilson et al., 2009
		0.58	0.42	IL	2002	Kelly et al., 2008
		0.41	0.59	ON	n/a	Haworth et al., 2020
C676A	1	0.99	0.01	IL	2002	Kelly et al., 2008
		0.98	0.02	AB, SK	2002,1996	Wilson et al., 2009
		0.97	0.03	WI	2002	Johnson et al., 2006
		0.96	0.04	ON	n/a	Haworth et al., 2020

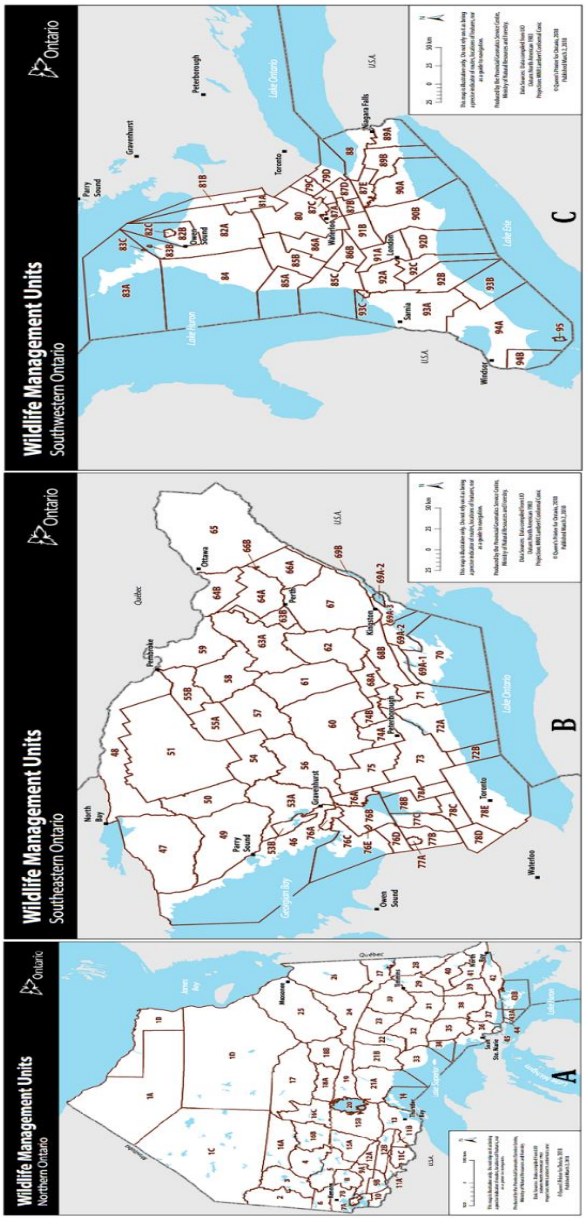
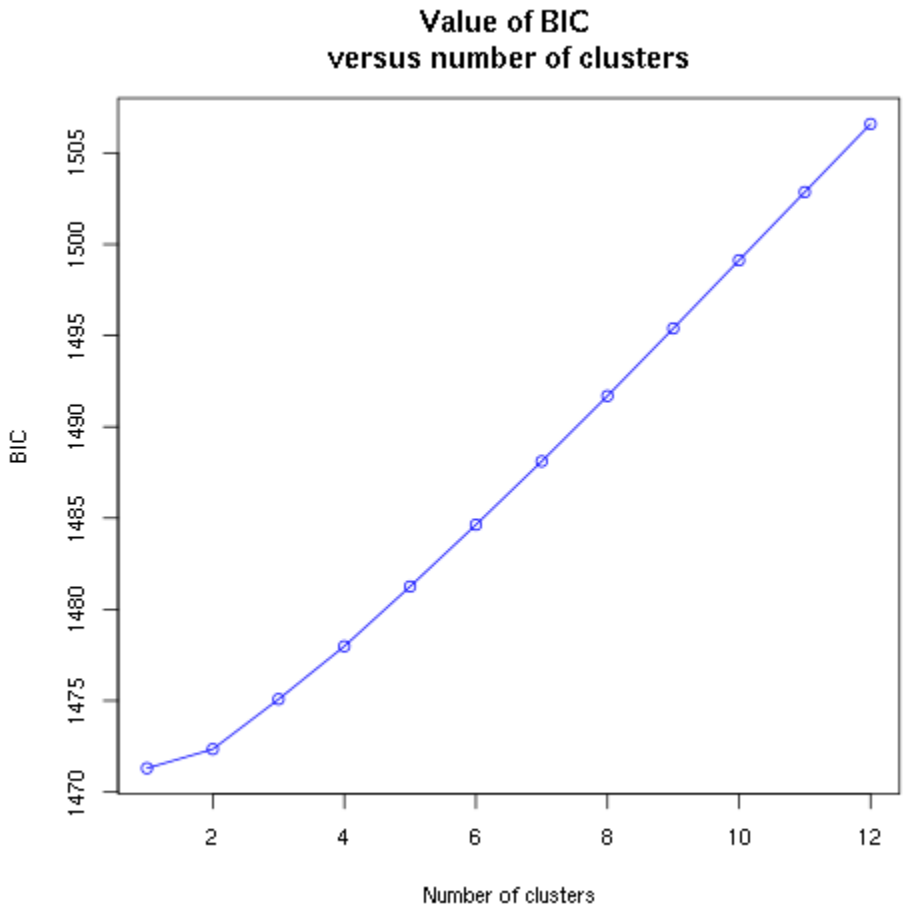


Figure S1. The Canadian province of Ontario as managed by the Ontario Ministry of Natural Resources and Forestry. There are three broad regions Ontario is managed by: (A) Northern Ontario, (B) Southeastern Ontario, and (C) Southwestern Ontario. Collectively (B) and (C) form Southern Ontario. Outlined in red are the wildlife management units designated within each broad region.



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839 **Figure S2**. The Bayesian information criterion (BIC) from a population cluster identification
840 using successive K-means cluster assignment on the reduced representation white-tailed
841 deer genome from identified one cluster as optimal.

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