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Prion Protein Gene (*PRNP*) Sequences Suggest Differing Vulnerability to Chronic Wasting Disease for Florida Key Deer (*Odocoileus virginianus clavium*) and Columbian White-Tailed Deer (*O. v. leucurus*)

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Abstract

Chronic wasting disease (CWD) is a fatal, highly transmissible spongiform encephalopathy caused by an infectious prion protein. CWD is spreading across North American cervids. Studies of the prion protein gene (*PRNP*) in white-tailed deer (WTD; *Odocoileus virginianus*) have identified non-synonymous substitutions associated with reduced CWD frequency. Because CWD is spreading rapidly geographically, it may impact cervids of conservation concern. Here, we examined the genetic vulnerability to CWD of 2 subspecies of WTD: the endangered Florida Key deer (*O. v. clavium*) and the threatened Columbian WTD (*O. v. leucurus*). In Key deer (n = 48), we identified 3 haplotypes formed by 5 polymorphisms, of which 2 were non-synonymous. The polymorphism c.574G>A, unique to Key deer (29 of 96 chromosomes), encodes a non-synonymous substitution from valine to isoleucine at codon 192. In 91 of 96 chromosomes, Key deer carried c.286G>A (G96S), previously associated with substantially reduced susceptibility to CWD. Key deer may be less genetically susceptible to CWD than many mainland WTD populations. In Columbian WTD (n = 13), 2 haplotypes separated by one synonymous substitution (c.438C>T) were identified. All of the Columbian WTD carried alleles that in other mainland populations are associated with relatively high susceptibility to CWD. While larger sampling is needed, future management plans should consider that Columbian WTD are likely to be genetically more vulnerable to CWD than

many other WTD populations. Finally, we suggest that genetic vulnerability to CWD be assessed by sequencing *PRNP* across other endangered cervids, both wild and in captive breeding facilities.

Subject Area: Conservation genomics & biodiversity
Keywords: cervids, endangered species, prion, transmissible spongiform encephalopathy

Chronic wasting disease (CWD) is a highly transmissible neurodegenerative spongiform encephalopathy (TSE), caused by prions (Williams and Young 1980; Belay et al. 2004). Prions are infectious proteins composed of the same amino acids as the normally folded cellular prion protein (PrP^{C}), but with an abnormal, yet stable, altered protein structure (Belay et al. 2004). This abnormal structure renders the prion protein infectious (PrP^{CWD}). The infectious protein (PrP^{CWD}) binds to PrP^{C} inducing conformational changes to additional abnormal PrP^{CWD}, destroying the standard function of the normal protein in the process and rendering it inactive (Belay et al. 2004). In the course of the infection, the infectious prions build up in the nervous system, causing a neurodegenerative process that leads to numerous cavities in brain tissue that are sponge-like in appearance. Prion diseases cause a progressive and irreversible deterioration of the organism's nervous system caused by insoluble aggregates that are resistant to protease degradation and thus are toxic to cells. This process ultimately leads to death, as there is no known treatment or cure for TSEs (Williams and Young 1980; Belay et al. 2004).

CWD has spread geographically across the populations of different cervid species in North America after being originally characterized in the 1960s in Colorado (Williams and Young 1980; Williams and Young 1982; Rivera et al. 2019). CWD has also recently spread to wild cervid populations in Finland, Norway, and Sweden, and a few captive populations in South Korea (Richards 2020). CWD is a potential threat to both captive and wild deer because this highly contagious disease can spread via infected bodily fluids such as saliva, urine, blood, and semen (Mathiason et al. 2006; Haley et al. 2009; Haley et al. 2011; Kramm et al. 2019). Natural and human-made mineral licks can aggregate cervids in high densities and increase the risk of direct transmission from infected individuals to healthy individuals (Plummer et al. 2018). In addition to the threat of exposure to CWD by direct transmission from infected animals, there is evidence that PrP^{CWD} can persist long-term in the environment. PrP^{CWD} can be sequestered by plants and remain infectious in soil particles for years, although certain soil characteristics can favor degradation of the prion protein (Nichols et al. 2009; Bartelt-Hunt and Bartz 2013; Kuznetsova et al. 2014; Kuznetsova et al. 2018). Within North America, CWD has spread to free-ranging cervid populations in at least 24 states in the United States and 2 provinces in Canada (CDC 2020); captive populations positive for CWD have been found in an additional 2 states and a Canadian province (Richards 2020). The US Fish and Wildlife Service (USFWS) and other US federal and state agencies are actively managing, monitoring, and attempting to reduce the spread of CWD (Bibb et al. 2010; Manjerovic et al. 2014; Staletovich 2018). CWD may even cause population declines after becoming endemic in a cervid population (Edmunds et al. 2016).

Variation in the prion protein gene (*PRNP*) has been associated with differences in the frequency of CWD among cervids. One study sequenced *PRNP* in 2433 white-tailed deer (WTD) in Illinois and southern Wisconsin (nominally *Odocoileus virginianus borealis*, although these populations were heavily impacted by translocations from outside the region) (Smith 1991; Kelly et al. 2008; Brandt et al. 2015; Brandt et al. 2018). Two *PRNP* haplotypes, designated haplotype C and haplotype F, are relatively less common in CWD positive deer than in CWD negative deer (Brandt et al. 2015; Brandt et al. 2018). The frequency of haplotype C was 0.328 in CWD-negative deer, but only 0.011 in CWD-positive deer. The frequency of haplotype F was 0.108 in CWD-negative deer, and only 0.0018 in CWD-positive deer (Brandt et al. 2015; Brandt et al. 2018). Each of the 2 haplotypes displays a non-synonymous SNP when compared to the most common haplotype. Haplotype C carries a non-synonymous substitution from G to A at nucleotide position 286 (c.286G>A), which encodes a serine (S) instead of the more common glycine (G) at codon 96 (G96S) (Brandt et al. 2015); while haplotype F carries a non-synonymous substitution at nucleotide position 285 (c.285A>C), which encodes a histidine (H) instead of the more common glutamine (Q) at codon 95 (Q95H) (Brandt et al. 2018). Both of these non-synonymous substitutions have been previously reported to be associated with reduced susceptibility to CWD in WTD (Johnson et al. 2006; Kelly et al. 2008). A study of *PRNP* polymorphisms in WTD reported that deer encoding QGS (95Q, 96G, and 138S) were disproportionately more common than deer encoding QSS (95Q, 96S, and 138S) among CWD positive deer (Johnson et al. 2003). Another study of *PRNP* polymorphisms reported that WTD encoding QGAS (95Q, 96G, 116A, and 138S) were disproportionately more common than deer encoding QSAS (95Q, 96S, 116A, and 138S) among CWD-positive deer (O'Rourke et al. 2004).

The geographic spread of CWD has raised concern about its potential to impact the health of endangered deer in the wild or in captive breeding programs (CDC 2020). Among WTD subspecies in the United States, 2 have been federally listed under the Endangered Species Act. The Florida Key deer (*Odocoileus virginianus clavium*) has the smallest body size of any subspecies of WTD. It is found in 20–25 islands of the lower Florida Keys (Hardin et al. 1984), from Bahia Honda Key to Sugarloaf Key, with the largest population in Big Pine Key (Folk 1991). Key deer evolved in an environment subject to hurricanes, fires, and droughts and lacking natural predators or competitors. In the 18th century, the Key deer population began to decline due to overhunting (Frank et al. 2003). They were almost extinct by 1950 when the National Key Deer Refuge was established. The subspecies was protected under the Endangered Species Preservation Act of 1966 and later, the US Endangered Species Act (ESA) in 1973 (Department of the Interior 1967; Hardin et al. 1984).

The Columbian WTD (*Odocoileus virginianus leucurus*) comprises the westernmost population of WTD. Historically, this subspecies ranged from the Puget Sound in Washington to the Umpqua River basin in Oregon and to the western slopes of the Cascade mountain range (Smith 1987; USFWS Oregon Fish and Wildlife Office 2013). Their populations declined in the late 19th century due to hunting and habitat loss and thus were placed under federal protection under the Endangered Species Preservation Act of 1966 and later, the US Endangered Species Act in 1973 (US Fish and Wildlife Service 2013). Currently, one population of Columbian WTD is found in mainland Washington near the lower Columbia River and the other population is found in Douglas County in southwest Oregon (USFWS Oregon Fish and Wildlife Office 2013).

Although CWD has not yet reached the range of either subspecies (Figure 1), genetic assessment of potential disease risk to these 2 endangered taxa of deer would be informative for developing and implementing management plans (Miller and Walter 2019; Robinson et al. 2019). Because genetic variation in *PRNP* is associated with substantial differences in the vulnerability to CWD of WTD, here we examine the entire coding region of *PRNP* in these 2 subspecies. We found that 94.8% of the Key deer (*O. v. clavium*) chromosomes carry a non-synonymous substitution that would be expected to make the subspecies less susceptible to CWD than previously genotyped mainland WTD populations. By contrast, genetic variation of *PRNP* in the Columbian WTD (*O. v. leucurus*) suggests that this subspecies would be genetically more susceptible to CWD outbreaks. This difference between the 2 subspecies is placed into the broader context of genetic management of deer to minimize the impact of CWD in wild and captive populations.

Materials and Methods

Deer Sampling

A total of 66 WTD samples were collected for use in the study. Twenty of the Key deer samples were collected under the authority of the USFWS) in the Florida Keys (Monroe County). Testicular or ovarian tissue was opportunistically collected from deer that were euthanized due to a New World screwworm (*Cochliomyia hominivorax*) outbreak in 2016, which killed approximately 10% of the Key deer population (US Fish and Wildlife Service 2016). The USFWS Office of Law Enforcement (OLE) National Fish and Wildlife Forensics Laboratory (NFWFL) provided DNA extracted from 32 Key deer, also collected in the Florida Keys (Monroe County). DNA extracts from 14 Columbian WTD samples were also provided by the USFWS National Fish and Wildlife Forensics Laboratory. These had been collected in Douglas County, Oregon (n = 11) and Wahkiakum County, Washington (n = 3). These samples were originally obtained by the USFWS OLE Forensics Laboratory in Ashland, Oregon, as part of law enforcement efforts and research projects under COSE & Federal Fish and Wildlife (Law Enforcement) permits, and were used with the permission of the USFWS. DNA was successfully amplified and sequenced from 61 of these samples (48 Key deer and 13 Columbian WTD) out of the 66 samples provided (Supplementary Table 1). The research in this project was conducted under the University of Illinois Institutional Animal Care and Use Committee protocol 18212.

DNA Amplification and Sequence Analysis

DNA was extracted from ovary and testes tissue using the Wizard Genomic DNA purification kit (Promega, Madison, WI). The incubation time for the samples was extended to 18 h and manufacturer's

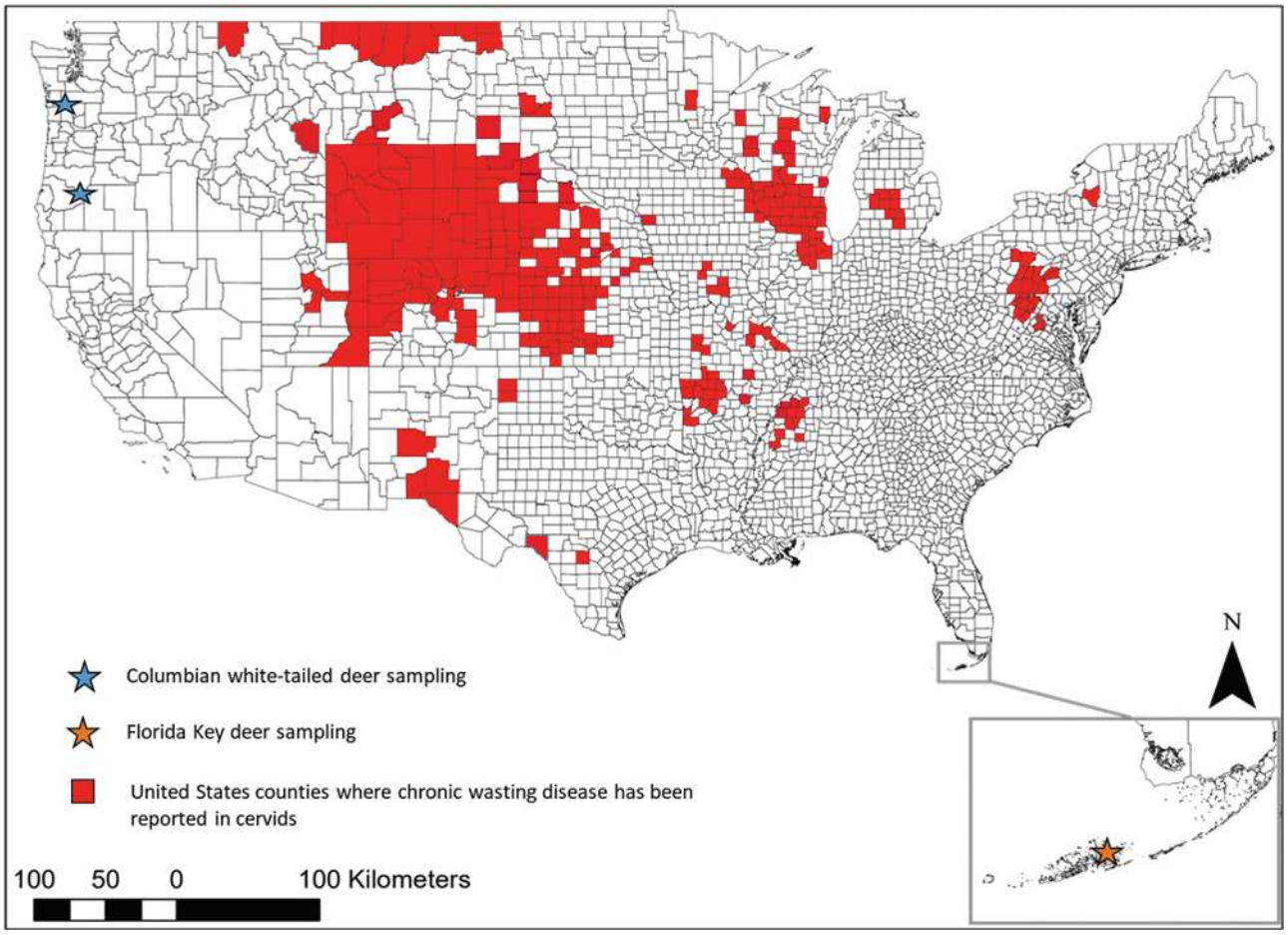

Figure 1. Map of the contiguous United States showing in red the counties from which chronic wasting disease has been reported in cervids as of January 2020 (CDC 2020). The blue stars indicate the location of sampling sites for Columbian white-tailed deer (*Odocoileus virginianus leucurus*). The orange star indicates the sampling location for the Florida Key deer (*O. v. clavium*). Map made using ArcGIS v10.7.1.

instructions were followed in all other aspects of the protocol. The DNA was amplified by PCR in 25.0 µl total volume, containing 1× PCR Buffer II (Applied Biosystems Inc.), final concentrations of 200 µM of each of the dNTPs, 1.5 mM $MgCl_2$, 0.04 units/µl of AmpliTaq Gold DNA Polymerase (Applied Biosystems Inc.) and 0.4 µM of each oligonucleotide primer (listed below). The *PRNP* gene in cervids consists of 3 exons, with only the third exon containing the coding sequence (771 bp). The forward primer 223 (5′-ACACCCTCTTTATTTTGCAG-3′) designed in intron 2 and the reverse primer 224 (5′-AGAAGATAATGAAAACAGGAAG-3′) located in 3′ untranslated region were used to amplify and sequence an 830 bp region including the complete coding region of *PRNP*, which is encoded by exon 3 (O'Rourke et al. 2004). Primers *PRNP*-IF (5′-ATGCTGGGAAGTGCCATGA-3′) and *PNRP*-IR (5′-CATGGCATTCCCAGCAT-3′) were used as additional internal primers for sequencing (Ishida 2020). Primers 223 and 224 were specifically designed to amplify the functional *PRNP* paralog, and to avoid the pseudogene (O'Rourke et al. 2004). PCR conditions were 95°C for 10 min for the initial denaturing; 5 cycles of 95°C for 30 s, 56°C for 30 s, and 72°C for 1 min; and 40 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 1 min; with a final extension of 7 min at 72°C. PCR amplification was confirmed using gel electrophoresis with a 1.0% agarose gel. All successful amplification products were enzyme-purified (Hanke and Wink 1994) and then sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (ABI). Sequences were generated in both directions with 1.0 µl of purified PCR product and 0.12 µM primers and were resolved on an ABI 3730XL DNA Sequencer at the Keck Center for Functional and Comparative Genomics at the University of Illinois at Urbana-Champaign. Sequences were visually examined, edited and concatenated using the Sequencher software 5.4.6 (Gene Codes Corporation, Ann Arbor, MI).

DNA Sequence Analysis

Using unphased data, haplotypes were inferred separately for each of the 2 subspecies using the program DnaSP utilizing Phase v2.1 (Stephens et al. 2001; Librado and Rozas 2009); 10 000 iterations were completed with 1000 burn-in iterations. Haplotype identities were verified using NCBI Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Haplotype sequences were aligned, open reading frames were confirmed and sequences were translated using MEGA X v.10.1 (Tamura et al. 2007). A median-joining network was generated and illustrated with PopART under default parameters (Bandelt et al. 1999; Leigh 2015). Confidence intervals for allele and haplotype frequencies in each population were calculated (Hazra 2017) using the following equation:

$$p \pm z\sqrt{\frac{p(1-p)}{n}}$$

To account for the difference in sample size between the Key deer and the Columbian WTD, we randomly resampled a subset of Key deer using RESEARCH RANDOMIZER (https://www.randomizer.org/). Thirteen Key deer unphased sequences (the same number as the sample size for Columbian WTD) were randomly chosen and then phased, with haplotypes inferred. This was repeated 10 times.

Results

Florida Key Deer

The complete coding region of the *PRNP* was sequenced in 48 Key deer. Using the software DnaSP (Librado and Rozas 2009), haplotypes were inferred from the unphased sequences. Within the coding region of *PRNP* in Key deer, 3 haplotypes and 5 SNPs were identified (Table 1). The haplotypes detected among the 48 Key deer samples were designated OVC1, OVC2, and OVC3.

Haplotype OVC1 was detected in 62 of 96 Key deer phased sequences (i.e., chromosomes) examined, and thus has the highest frequency among the samples (Figure 2). A synonymous substitution, c.499C>A, was found in haplotype OVC1 but not OVC2 or OVC3 (Table 1). The DNA sequence of haplotype OVC1 matches the sequence of a Texas WTD (*Odocoileus virginianus texanus*) in GenBank (amino acid: XP_020739306, nucleotide: XM_020883647), which may suggest that this haplotype was present in the founder population.

Haplotype OVC2 had the second-highest frequency among the samples. Importantly, in haplotype OVC2 (but not in OVC1 or OVC3), a non-synonymous substitution, c.574G>A, was detected, which encodes for an amino acid substitution from valine to isoleucine at codon 192. This haplotype was carried by 29 out of the 96 Key deer chromosomes, a frequency of 0.302 ± 0.092 (95% confidence interval [CI]).

Table 1. *PRNP* single nucleotide polymorphisms found in white-tailed deer subspecies

White-tailed deer subspecies	*PRNP* haplotype	Nucleotide position							
		285	286	303	438	499	555	574	*n*
Northern white-tailed deer	A	A	G	G	C	A	C	G	–
Odocoileus virginianus borealis	C	.	**A***	.	.	.	T	.	–
	F	**C***	.	.	.	.	.	.	–
Florida Key deer	OVC1	.	**A***	.	.	C	T	.	62
Odocoileus virginianus clavium	OVC2	.	**A***	.	.	.	T	A*	29
	OVC3	.	.	A	.	.	.	.	5
Columbian white-tailed deer	OVL1	.	.	.	.	.	.	.	20
Odocoileus virginianus leucurus	OVL2	.	.	.	T	.	.	.	6

Single nucleotide polymorphisms (SNPs) within the prion protein gene (*PRNP*) are compared across 3 northern white-tailed deer (*O. v. borealis*) haplotypes (Brandt et al. 2015, Brandt et al. 2018), and haplotypes in the Florida Key deer (*O. v. clavium*) and the Columbian white-tailed deer (*O. v. leucurus*). Only sites polymorphic between or within subspecies are shown. Nucleotides matching those in haplotype A are shown as dots, while the character state is shown for those that differ. Asterisks indicate non-synonymous SNPs relative to haplotype A. Non-synonymous SNPs previously reported to be associated with significantly reduced occurrence of CWD in white-tailed deer are in boldface and shaded. The number of chromosomes (*n*) carrying each haplotype is listed.

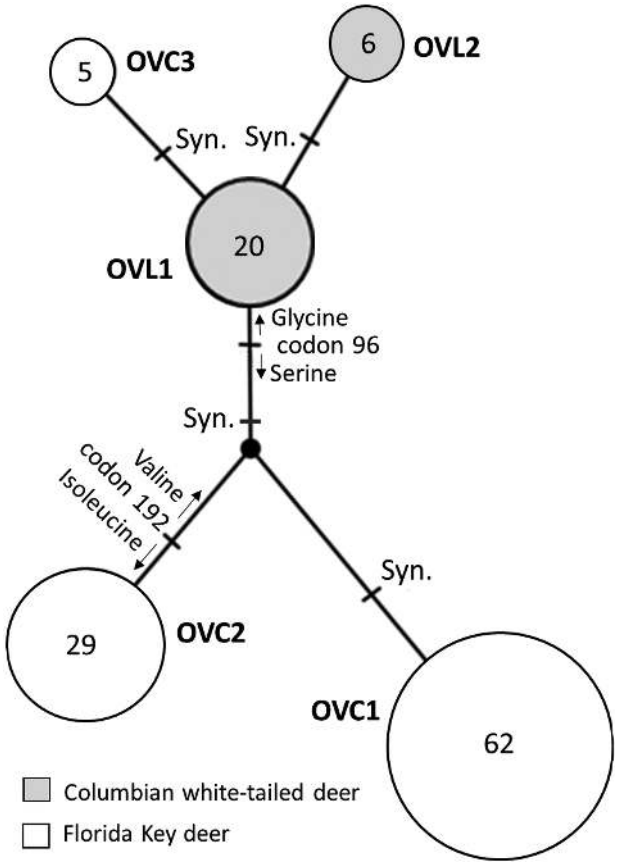

Figure 2. Median-joining network showing relationships among haplotypes of *PRNP* among Florida Key deer (*Odocoileus virginianus clavium*; unshaded circles) and Columbian white-tailed deer (*O. v. leucurus*; shaded circles). Each circle represents a distinct haplotype; each hatch mark on the branches separating circles represents a mutation. The size of each circle is proportional to the number of chromosomes carrying the haplotype, which is also listed within the circle. The designation for each haplotype is indicated in bold font. "Syn" denotes a synonymous nucleotide substitution. For non-synonymous mutations, the 2 encoded amino acids are shown on either side of the hatch mark. In white-tailed deer, serine at codon position 96 is associated with a reduced vulnerability to CWD relative to deer with glycine at codon position 96.

Two previously reported SNPs (Johnson et al. 2003; O'Rourke et al. 2004; Johnson et al. 2006; Kelly et al. 2008; Brandt et al. 2015; Brandt et al. 2018), a non-synonymous substitution, c.286G>A, and a synonymous substitution, c.555C>T, were both found in haplotypes OVC1 and OVC2 (but not in OVC3) and thus were present in 91 out of 96 (0.948) chromosomes. Allele 286A present in OVC1 and OVC2 encodes a serine instead of glycine at codon 96 (Table 2). The 95% CI for the frequency of allele 286A in the Key deer population was calculated as 0.90–0.99.

Haplotype OVC3, which carries a synonymous substitution, c.303G>A, was present in 5 out of 96 chromosomes (0.052 ± 0.0445 [95% CI]) (Figure 2). This is a novel polymorphism, and the DNA sequence of Key deer haplotype OVC3 has not been previously reported. However, haplotype OVC3 does encode the same amino acid sequence as many haplotypes present in Illinois and Wisconsin WTD, including haplotype A (Table 2), the most common haplotype in that region (Brandt et al. 2015; Brandt et al. 2018).

Columbian WTD

Two haplotypes, designated OVL1 and OVL2, were detected among the 13 Columbian WTD samples sequenced. A single synonymous substitution, c.438C>T, separates haplotype OVL1 from OVL2 (438T in OVL1, 438C in OVL2). Both sequences have been previously reported in WTD (Raymond et al. 2000; Zink et al. 2020).

The haplotype OVL1 was present in 20 of 26 chromosomes (0.769 ± 0.141 [95% CI]) (Figure 2). The DNA sequence of OVL1 is identical to the overlapping region of haplotype A (GenBank accession number: MG856905). Haplotype A is the most common haplotype among WTD in Illinois and Wisconsin (Table 1) (Brandt et al. 2015; Brandt et al. 2018). Haplotype OVL2 was present in 6 out of 26 chromosomes (0.231 ± 0.141 [95% CI]). The DNA sequence of OVL2 is an exact match to the overlapping region of the previously reported "Haplotype E" which is the fifth most common haplotype among WTD in Illinois and Wisconsin (GenBank accession number: MG856909) (Brandt et al. 2015; Brandt et al. 2018), and is also reported in other studies (Raymond et al. 2000; Zink et al. 2020). Haplotype E encodes the same amino acid sequence as haplotype A (Table 1).

Both haplotypes OVL1 and OVL2 encode for the same amino acid sequence, which is not associated with reduced vulnerability to CWD (Table 1). In the mule deer (*Odocoileus hemionus*), a sequence that encodes the same amino acid sequences as OVL1 and OVL2 has been reported (Genbank accession number: AAC33174).

To examine the impact of sample size differences between the Key deer and the Columbian WTD on the detected haplotype numbers, we randomly resampled subsets of 13 Key deer. The number of haplotypes inferred averaged 2.7 and was close to 3; the haplotype number detected in the 48 Key deer. Alleles associated with reduced frequency of CWD were present in each of the 10 sub-samples of 13 Key deer, whereas no alleles associated with reduced frequency of CWD were present in our sample of 13 Columbian WTD.

Discussion

Among the polymorphisms identified across the full coding region of *PRNP* in the 2 separately assessed subspecies, only Key deer encode a non-synonymous mutation (a valine to isoleucine substitution) at codon 192, which has not been reported in mainland WTD (Figure 2). The valine to isoleucine substitution at codon 192 is a conservative amino acid replacement because both amino acids are aliphatic and hydrophobic, although valine is smaller (Betts 2003). Further investigation into this non-synonymous mutation would be needed to identify any effects on the structural stability and ease of conformational change at the encoded PrP.

A study published as ours was under review also found this non-synonymous mutation at codon 192 in Key deer (Zink et al. 2020). However, unlike the study by Zink et al., we did not find 96S to be fixed in Key deer, but detected a low frequency of 96G in Key deer (in Haplotype OVC3). This difference may be due to sample size, as we examined 48 Key deer whereas Zink et al. (2020) sampled 15 Key deer.

The large majority (91 of 96) of Florida Key deer chromosomes examined had a serine at codon 96, which is associated with lower susceptibility to CWD in WTD (Johnson et al. 2003; O'Rourke et al. 2004; Kelly et al. 2008; Johnson et al. 2011; Brandt et al. 2015; Brandt et al. 2018). At codon 96, Key deer haplotypes OVC1 and OVC2 matched the previously described QSS (95Q, 96S, and 138S) (Johnson et al. 2003) and QSAS (95Q, 96S, 116A, and 138S)

Table 2. Non-synonymous variation in white-tailed deer subspecies and mule deer

Deer population	Designation	Codon position, encoded amino acid					States	References
		95	96	116	138	192		
Mainland white-tailed deer	Haplotype A	Q	G	A	S	V	Illinois, Wisconsin	Brandt et al. 2015; Brandt et al. 2018
Odocoileus virginianus	QGAS	Q	G	A	S	V	Nebraska	O'Rourke et al. 2004
	QGS	Q	G	A	S	-	Wisconsin	Johnson et al. 2003
	Haplotype C	Q	**S**	A	S	V	Illinois, Wisconsin	Brandt et al. 2015; Brandt et al. 2018
	QSAS	Q	**S**	A	S	-	Nebraska	O'Rourke et al. 2004
	QSS	Q	**S**	A	S	V	Wisconsin	Johnson et al. 2003
	Haplotype F	**H**	G	A	S	V	Illinois, Wisconsin	Brandt et al. 2015; Brandt et al. 2018
Florida Key deer	OVC1	Q	**S**	A	S	V	Florida	This Study
O. v. clavium	OVC2	Q	**S**	A	S	I*	Florida	This Study
	OVC3	Q	G	A	S	V	Florida	This Study
Columbian white-tailed deer	OVL1	Q	G	A	S	V	Oregon, Washington	This Study
O. v. leucurus	OVL2	Q	G	A	S	V	Oregon, Washington	This Study
Mule deer	N/A	Q	G	A	S	V	Colorado	O'Rourke et al. 1998
Odocoileus hemionus								

Haplotype A is the most common *PRNP* haplotype among white-tailed deer in Illinois; the same amino acids are encoded by other common Illinois haplotypes, notably haplotypes B, D, E, and G (not shown). Boldface and shading indicate non-synonymous variants associated with reduced susceptibility to CWD; a dash indicates missing information. Note that the Florida Key deer haplotype OVC1 does not have non-synonymous differences when compared to haplotype C, QSAS, or QSS deer in the mainland population (associated with reduced vulnerability to CWD). The asterisk indicates a non-synonymous mutation found in OVC2 in Florida Key deer. OVC3 in the Florida Key deer and OVL1 and OVL2 in the Columbian white-tailed deer do not have non-synonymous differences when compared to haplotype A, QGAS or QGS. Thus, none of the Columbian white-tailed deer carried a SNP associated with reduced susceptibility to CWD. The Columbian black-tailed deer (*O. h. columbianus*), a subspecies of the mule deer, is sympatric with the Columbian white-tailed deer. PrP with amino acid sequences identical to those of the Columbian white-tailed deer have been reported in mule deer from other subspecies. Codons 116 and 138 are not variable in this listing, but are shown because these positions have non-synonymous polymorphisms in some haplotypes of *PRNP* not listed here.

(O'Rourke et al. 2004), both of which are associated with reduced susceptibility to CWD in WTD (Table 2). Almost all Key deer carry this protective 96S allele, which has a much lower frequency in mainland populations (Johnson et al. 2003; O'Rourke et al. 2004; Kelly et al. 2008; Johnson et al. 2011; Brandt et al. 2015; Brandt et al. 2018). Should CWD reach the subspecies, Key deer are likely to be less vulnerable than mainland WTD populations to CWD.

By contrast, none of the Columbian WTD examined to date carry protective SNPs, and all encode the same protein variant (Table 2). All sequences coded for a glutamine at codon 95 and a glycine at codon 96, both of which have been associated with higher vulnerability to CWD in previous studies (Table 2) (Johnson et al. 2003; O'Rourke et al. 2004; Kelly et al. 2008; Johnson et al. 2011; Brandt et al. 2015; Brandt et al. 2018). WTD with this amino acid combination demonstrate higher susceptibility to CWD than those encoding a histidine at codon 95 and/or a serine at codon 96 (Table 2) (Johnson et al. 2003; O'Rourke et al. 2004; Kelly et al. 2008; Johnson et al. 2011; Brandt et al. 2015; Brandt et al. 2018). This suggests that if Columbian WTD were exposed to CWD they would be extremely susceptible to the disease, even more vulnerable than deer populations located in currently affected states such as Illinois or Wisconsin, in which a substantial proportion of WTD carry *PRNP* alleles associated with reduced frequency of CWD (Brandt et al. 2015; Brandt et al. 2018). However, our survey included only 13 samples. Larger sampling of the 2 populations of Columbian WTD would be required to verify that there is a dearth of alleles associated with reduced susceptibility to CWD in the subspecies.

North American elk (*Cervus canadensis*) and the Columbian black-tailed deer (*Odocoileus hemionus columbianus*) are cervid taxa sympatric with the Columbian WTD; both are potentially susceptible to CWD (Miller and Williams 2004; Forrester and Wittmer 2013), and thus pose the risk of interspecies transmission of CWD to the Columbian WTD. The Columbian black-tailed deer is known to hybridize with the Columbian WTD (Latch et al. 2011), further increasing the future risk of interspecies transmission of CWD (Gavin and May 1988; Bradley et al. 2003; Hopken et al. 2015). The Columbian black-tailed deer is a subspecies of the mule deer (*Odocoileus hemionus*). In other mule deer populations, *PRNP* sequences have been reported that encode the same PrP amino acid sequence found in the Columbian WTD (Table 2), this PrP similarity may potentially increase the risk of interspecies transmission. However, it is possible that non-synonymous variation in *PRNP* may differ across subspecies of mule deer, and previous research on mule deer has detected mutations in *PRNP* associated with differences in susceptibility to CWD (Jewell et al. 2005; Wilson et al. 2009; Wolfe et al. 2014) that are different from the *PRNP* mutations that affect CWD in WTD. Thus, local populations of sympatric cervids would need to be separately assessed to determine their own genetic vulnerability to CWD, and the risk that sympatric taxa pose for inter-species transmission of CWD to the Columbian WTD. There is also a possibility of intra-species transmission of CWD to the Columbian WTD, because its range is contiguous with that of the northwestern subspecies of WTD (*O. v. ochrourus*). Overall, the risk of CWD transmission via other WTD subspecies or other cervid species (Miller and Williams 2004; Forrester and Wittmer 2013; Manjerovic et al. 2014; Hopken et al. 2015) should be an important management consideration, especially given that Columbian WTD are likely to be extremely vulnerable to CWD should the disease enter the population.

Both Key deer and Columbian WTD have experienced substantial population declines resulting in their listing under the federal Endangered Species Act (Hardin et al. 1984; USFWS Oregon Fish and Wildlife Office 2013). Despite federal protection, Key deer recovery has been challenging. A deadly screwworm outbreak in 2016

and the locally devastating Hurricane Irma in 2017 reduced the population of Key deer to fewer than 1000 individuals (Johnson et al. 2003; Hires 2017; Nobel 2017). For these reasons, Key deer represent a particularly pressing conservation concern and proactive assessments about potential risks to their population would be informative to population managers. In contrast, the Douglas County, Oregon, Columbian WTD population has rebounded, with a current population of around 5000 (USFWS Oregon Fish and Wildlife Office 2013). In 2003 its federal status was changed from endangered to threatened (US Fish and Wildlife Service 2013). Additionally, in 2013, following a 5-year review, the US Fish and Wildlife Service recommended that the status of the Lower Columbia River population, now numbering ca. 1300 individuals (USFWS Oregon Fish and Wildlife Office 2013), also be changed from endangered to threatened (US Fish and Wildlife Service 2013). While currently the Columbian WTD populations appear to be rebounding, delisting, and new hunting allowances suggest that these populations should still be continuously monitored (Ricca et al. 2002).

CWD has not yet spread to Florida, Oregon, or Washington, with the nearest reported cases of CWD being at least 900 km from each of the populations (Figure 1). However, CWD is a potential risk to both of these subspecies as the disease continues to spread into previously unexposed cervid populations in North America. Increased sampling of cervids for early disease detection and strict management of cervids being transferred across state lines are among the measures being taken by the Association of Fish and Wildlife Agencies to reduce the spread of CWD (Gillin and Mawdsley 2018). Measures such as culling diseased animals, culling males only, or increasing overall hunting have been shown to decrease CWD prevalence, but these measures can be expensive to implement and have varying success in decreasing CWD once it affects a population (Uehlinger et al. 2016; CDC 2020).

To our knowledge, this study is the first to assess genetic vulnerability to CWD in Columbian WTD. It also greatly increases the number of Key deer sequences. *PRNP* genetic variation, and its implications for CWD vulnerability in WTD populations, can be taken into consideration by wildlife managers. The survey of *PRNP* genetic variation could potentially be extended to other endangered cervid taxa in the wild or in captive breeding programs, in order to assess their genetic vulnerability to CWD. For endangered deer species that show variation in *PRNP*, selective breeding or translocation of deer from more CWD resistant populations (as long as the deer are not taxonomically distinctive) could be used to reduce the proportion of deer that carry alleles associated with higher vulnerability to CWD and increase the proportion of deer alleles associated with lower vulnerability to CWD. Such a strategy would gradually increase the genetic health of deer stocks against CWD. Our results suggest that, even within a species, some populations may be much more vulnerable genetically than others to CWD, highlighting the continued importance of efforts to reduce the impact and spread of CWD.

Supplementary Material

Supplementary material is available at *Journal of Heredity* online.

Supplementary Table 1. Florida Key deer (*Odocoileus virginianus clavium*) and Columbian white-tailed deer (*O. v. leucurus*) sample information. Table of samples used, showing sample IDs, subspecies of the samples and the states from which samples were collected. Samples with "original samples IDs" that begin with "2016" were from Florida Key deer that died or had to be euthanized due to a screwworm outbreak in 2016, and were collected under the authority of the U.S. Fish and Wildlife Services. All other samples were provided by the USFWS National Fish and Wildlife Forensics Laboratory.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Data Availability

PRNP haplotype full coding sequences for Key deer and Columbian white-tailed deer have been deposited in GenBank under accession numbers MT944345-MT944349. Sample information for the study is presented in Supplementary Table 1.

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