

Molecular assessment of translocation and management of an endangered subspecies of white-tailed deer (*Odocoileus virginianus*)

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Abstract Translocations are an effectual management strategy for the reestablishment and reconnection of endangered populations and species. However, knowledge about the evolution and ecology of the populations and species of interest are critical so that informed decisions can be made about source populations and reestablishment areas. We employed 614 base pairs of the mitochondrial control region and 15 microsatellite loci to investigate genetic variation, contemporary connectivity, and inter-specific hybridization in the two remaining populations of the endangered Columbian white-tailed deer (*Odocoileus virginianus leucurus*) through comparisons with the closest subspecies, *O. v. ochrouros*. Our data revealed the dubious taxonomic status of *O. v. leucurus*, and that *O. virginianus* in the Pacific Northwest originated from a single historic gene pool. Further the results identified that populations are currently genetically isolated and depauperate, and uncovered historic introgression with *O. hemionus columbianus*. These results suggest that translocations are a viable approach for reestablishing populations throughout

the historic range to increase genetic diversity in the fragmented populations. Despite the taxonomic ambiguity, our study revealed the presence of unique genetic variation within each population which supports ongoing conservation efforts.

Keywords Translocations · Genetic diversity · Subspecies · Introgression · Pacific Northwest

Introduction

Development of proactive conservation and management plans that consider both ecological and evolutionary processes are important first steps towards halting the decline and facilitating the recovery of many threatened and endangered (T and E) species. Translocations can be part of these broader conservation and management strategies and typically consist of two goals: (1) reestablish populations within a species' historic range (reintroductions); and (2) facilitate gene flow between disconnected populations (augmentation) (IUCN 1987). The aim of reintroductions is to increase the total number of populations and geographic distribution of a species thus lessening the chance that an isolated stochastic event could cause complete species extinction. Augmentations on the other hand are expected to increase the effective population size (N_e) of isolated populations which can restore and retain genetic variation to pre-bottleneck levels (Hedrick and Fredrickson 2010). Many once wide-ranging species are now relegated to small islands of suitable habitats thus translocations are integral to maintaining genetic diversity and decreasing extinction risk through enhanced resistance and resilience to changing environments (Shaffer 1981; Weeks et al. 2011). There are numerous examples of translocations

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countering small population effects by reestablishing historic population numbers (e.g., Deyoung et al. 2003), and historic levels of genetic diversity (e.g., Bouzat et al. 2009).

A major impediment for conservation and management is that many species, subspecies, and populations are protected as entities described by limited morphological and geographic data generated in the nineteenth and early twentieth centuries, which often do not correlate with true phylogenetic units (Dubois 2003). Yet, one primary goal of conservation and management is to protect independent evolutionary units (Soltis and Gitzendanner 1999). When uncertainty exists about taxonomic status, conservation and management efforts could be diluted if populations are incorrectly designated as endangered, or extinction may be possible when a unique population is lumped with an abundant lineage. Polytypic species that are currently classified as several subspecies based on original morphological descriptions and have not been investigated with modern methods are thus prime candidates for re-evaluation of the validity of each subspecific taxon (Dubois 2003).

Accurate taxonomy that reflects phylogenetic relationships, whether inter- or intraspecific, helps facilitate management decisions which is critical in the context of translocations. The International Union for the Conservation of Nature (IUCN) recommends that translocation source populations be the same “race” as the recipient populations (IUCN 1987). Race however was not defined and is not a recognized taxonomic rank in the International Code for Zoological Nomenclature (ICZN), leading some to suggest that donor populations be of the same species and collected within geographic proximity to the recipient population (Hedrick and Fredrickson 2010; Vergeer et al. 2004). Others, however, have questioned the geographic proximity/close evolutionary relationship argument because the populations nearby may be genetically depauperate themselves due to the same factors that caused decline of the population of concern (Weeks et al. 2011). Populations that are still widespread and abundant may have unique variation that can be used to increase genetic diversity of depauperate populations. The predominant concern regarding translocations is the disruption of local adaptations and reducing population fitness through outbreeding depression and genetic load (Edmands 2007; Storfer 1999). But others have countered that T and E populations may have already lost local adaptations due to small effective sizes and genetic drift (Lopez et al. 2009). Frankham et al. (2011) therefore developed a framework for evaluating the probability of outbreeding depression in cases without adequate time or resources available to perform breeding experiments. Their criteria were similar to the IUCN and were based on taxonomy, chromosomal

differences, recent gene flow, environmental differences, and time since population fragmentation. Herein, we undertake two steps of this process, using molecular data: (1) to test current taxonomic hypotheses regarding white-tailed deer subspecies [*O. virginianus* (Zimmerman)], and (2) to assess population connectivity (gene flow) to assist the evaluation of translocation of an endangered subspecies, the Columbian white-tailed deer [*Odocoileus virginianus leucurus* (Douglas)].

There are currently two recognized subspecies of *O. virginianus* in the Pacific Northwest, USA (PNW): Columbian white-tailed deer (*O. v. leucurus*) and the Northwest white-tailed deer (*O. v. ochrourus* Bailey). Douglas (1829) originally described *O. v. leucurus* as a distinct species (*O. leucurus*) based on geographic location and morphometrics of a single specimen from what is now Douglas County, Oregon. The basis for his classification was geographic isolation, pelage color, smaller body size, antlers, and skeletal structure than other *O. virginianus* subspecies. *Odocoileus v. leucurus* was once considered abundant along the northern Pacific coast but quickly declined by the mid-nineteenth century (Bailey 1936; Cowan 1936; Douglas 1829). The historic range of *O. v. leucurus* is estimated as extending from Puget Sound in the north to the Willamette Valley in southern Oregon, and from the Pacific Coast east to The Dalles, Oregon with the Cascade Mountain Range as the eastern barrier (Cowan 1936; Livingston 1987). *Odocoileus v. leucurus* currently exists in two isolated populations, as multiple subpopulations on the banks and Islands of the Lower Columbia River in both Washington and Oregon, and in Douglas County, Oregon (Fig. 1; Gavin 1984; Smith 1985). Decline of the subspecies is most likely due to habitat alterations for agriculture and unregulated hunting (Brookshier 2004; Gavin 1978; Scheffer 1940; Smith 1985; Suring and Vohs 1979). The Cascades are the western barrier for *O. v. ochrourus* which currently occurs in the northeastern corner of Oregon, eastern Washington, and eastward into Idaho, Montana, and Wyoming (Peek 1984).

Until the early twentieth century biologists thought that the only extant *O. v. leucurus* were in Douglas County, Oregon (Gavin 1984). However, a second population was discovered about 340 km to the north along the Lower Columbia River (Scheffer 1940). This discovery ultimately led to the establishment of the Julia Butler Hansen National Wildlife Refuge for the Columbia White-Tailed Deer in 1972. Both populations were deemed endangered under the U.S. Endangered Species Act (ESA) in 1973 (Lower Columbia) and in 1978 (Douglas County) (Federal Registers 35 FR 13519; 48 FR 49244; 68 FR43647). Each population was later recognized as Distinct Population Segments (DPS) by the U.S. Fish and Wildlife Service (Federal Register 61 FR 4722). The Douglas County DPS

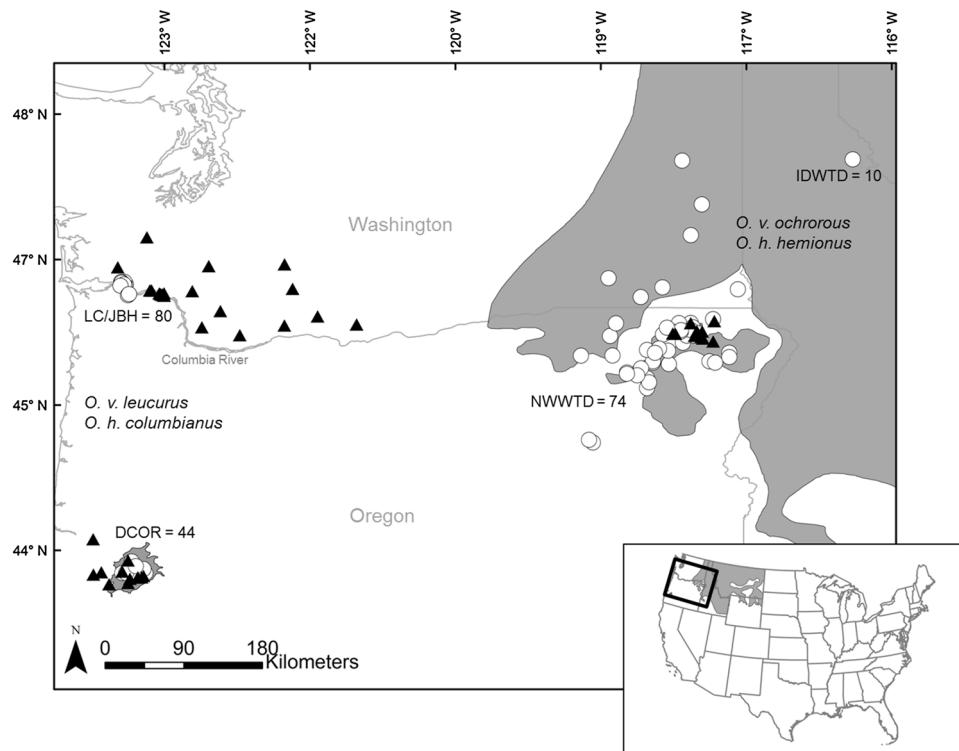


Fig. 1 Distribution and sampling map of *Odocoileus virginianus* and *O. hemionus* from the Pacific Northwest of the United States. The inset shows the sampling area (black box) for this study in relation to the continental U.S. The gray areas represent the putative range of *O. virginianus* in the Pacific Northwest. Sampling locations are designated by (open circle) for *O. virginianus* and (filled triangle) for *O. hemionus*. Each symbol can represent more than one individual captured at that location. Subspecies from each location were *O. v. leucurus* and *O. h. columbianus* along the Pacific coast and *O. v.*

ochrorous and *O. h. hemionus* from Eastern Oregon, Eastern Washington and Idaho (denoted on map). Abbreviations designate sampling locations for: Lower Columbia River/ Julia Butler Hansen Refuge (LC/JBH); Douglas County, Oregon (DCOR); Eastern Oregon and Washington (NWWTD) and Idaho (IDWTD). Wyoming sampling locations are not included in this map but are available from authors upon request. Sample sizes for each sampling location are for *O. virginianus* only

was removed from the Federal Endangered Species List in 2003 because the estimated population size met requirements outlined in the recovery plan (Federal Register 68 FR 43647). For at least the last 100 years, the two populations have remained isolated, which has raised concerns about low genetic diversity, inbreeding, and the resulting increased extinction risk. Remarkably, the Douglas County population recovery has been so successful that managers now have concerns about overpopulation leading to disease outbreaks and human/deer conflicts (T. Lum, personal observation). Due to the high population density within Douglas County, the Oregon Department of Fish and Wildlife (ODFW) collaborated with the U.S. Fish and Wildlife Service to develop and implement a plan, based on the results presented herein, for translocations of individuals from both populations of *O. v. leucurus*. The goals were to reestablish populations in recovered habitats within the historic range, reconnect the two populations to increase the genetic diversity, and reduce population overabundance in Douglas County.

The genetic relationships of *O. virginianus* in Oregon and southern Washington were previously investigated using allozyme loci and mitochondrial DNA restriction fragment length polymorphisms (RFLPs; Cronin 1991; Gavin and May 1988). The results suggested minimal genetic differences among populations, and that *O. v. leucurus* from Douglas County and *O. v. ochrorous* in northeastern Oregon experienced recent gene flow, or were both derived from a single recent founder event. Further, Gavin and May (1988) suggested that the only population that potentially warranted subspecific designation was the Lower Columbia River population; however, they were careful to note that genetic differences were based on allele frequencies at one locus and ultimately did not recommend a final taxonomic decision.

One potential problem with translocation programs arises when a potential source population has experienced hybridization or introgression with another species or subspecies. Introduction of such genetic diversity into an endangered stock could thus jeopardize the evolutionary

legacy of that lineage. It is well documented that black-tailed/mule deer [*O. hemionus* (Rafinesque)] and *O. virginianus* hybridize (Carr et al. 1986). Considering the Columbian black-tailed deer [*O. h. columbianus* (Richardson)] is sympatric with *O. v. leucurus* across the latter's distribution, introgression of *O. h. columbianus* DNA into populations of *O. v. leucurus* is a cause for concern (Cronin 1991; Gavin and May 1988). Clearly, further elucidation of the genetic relationships of *Odocoileus* spp. in the PNW is critical to aid and inform conservation and management goals.

The findings of Gavin and May (1988) and Cronin (1991), and the need to assess the practicality of a translocation strategy motivated us to evaluate the sub-specific status and connectivity of the two *O. v. leucurus* populations through a comparison with the geographically closest *O. v. ochrorous* population east of the Cascades (Fig. 1). We further investigated the genetic integrity of all three *O. virginianus* populations by evaluating hybridization with sympatric *O. hemionus*. We employed two fast evolving genetic markers, mitochondrial and microsatellite DNAs, and utilized phylogenetic and population genetic methods to accomplish our goals. The results from this study will provide wildlife managers with genetic information to assist with decisions regarding the practicality of translocations and other management strategies for *O. virginianus*.

Materials and methods

Sample collection

Odocoileus spp. samples were collected from Oregon, Washington, Idaho, and Wyoming (Fig. 1). Final sampling included 80 individuals from the Lower Columbia River region of Oregon and Washington (LC/JBH), 44 from Douglas County, Oregon (DCOR), and 77 from northeast Oregon and southeast Washington (NWWTD). *Odocoileus virginianus* from Idaho (= IDWTD; n = 10), Nebraska (NEWTD; n = 2), and Wyoming (= WYWTD; n = 3) were collected to serve as outgroups. We also sampled *O. h. columbianus* (BTD; n = 25) from Douglas County, Oregon and southern Washington, and *O. h. hemionus* [(Rafinesque) MD; n = 22] from northeast Oregon to include as outgroups and to evaluate hybridization (Gavin 1984; Gavin and May 1988). Most samples were collected as tissue from live deer captures or road kill and preserved in EDTA–DMSO buffer. Some of the samples were collected using DNA darts (Pneu-Dart, Inc., USA) which provided either small pieces of tissue or hair. Blood samples were collected from deer on Tenasillahe Island, Oregon on FTA cards (GE Healthcare, Piscataway, New Jersey, USA).

DNA isolation

Genomic DNA was extracted from skin, muscle, and FTA cards using the DNeasy Blood and Tissue Kit (Qiagen). For tissue samples, we followed the manufacturer's animal tissue protocol. The FTA card DNA extraction required slight alterations (see electronic supplementary material). Some dart samples contained only hair and were extracted with the Qiamp Micro Kit (Qiagen) following the forensic case work protocol.

Mitochondrial DNA sequences

DNA sequencing was accomplished by amplifying the hyper-variable region I (HVI) of the mitochondrial DNA control region using primers from Purdue et al. (2000) (see appendix for PCR conditions in electronic supplementary material). Amplification success was ascertained with 2 % agarose gels stained with ethidium bromide. Successful amplifications were purified using ExoSAP-IT (U.S. Biological, USA). Cycle sequencing reactions were performed in 10 μ l reactions with 1 μ l of purified PCR product, 1 μ M primer, 0.25 μ l BigDye v3.1, and 2.275 μ l 5 \times sequencing buffer [Applied Biosystems (ABI), USA]. Sequencing was performed on an ABI 3130xl genetic analyzer.

Microsatellite DNA genotyping

Seventeen microsatellite loci were amplified in four multiplex panels (Anderson et al. 2002) (Table A1 in electronic supplementary material for PCR conditions). All fragments were analyzed on an ABI 3130 Genetic Analyzer. Alleles were binned and scoring was manually evaluated using GENEMAPPER v. 4.0 (ABI). Fifteen microsatellite loci were used for final analyses, except the mean genetic diversity estimates which were based on 16 loci, due to problems with violations of assumptions of equilibria (see results).

Data analysis

Sequence data were edited and aligned using SEQUENCHER v4.9 (Gene Codes Corp., Ann Arbor, MI, USA). Haplotype (*h*) and nucleotide (π) diversities were calculated with DNASP v5.0 (Librado and Rozas 2009). Redundant haplotypes were removed for maximum likelihood (ML) analyses using ALTER (Glez-Peña et al. 2010) leaving a total of 52 haplotypes in the tree. For ML tree generation the evolutionary model that best fit our sequence data, TVM+I+G (parameters are listed in Table A2 in electronic supplementary material), was selected with the Akaike Information Criterion (AIC) in jMODELTEST (Posada 2008). The parameters estimated under this model were applied to the likelihood settings in PAUP* v. 4.0 (Swofford

2003). We rooted the tree with *O. h. columbianus* haplotypes. Branch support was quantified with bootstrapping (Felsenstein 1985) generated by PAUP*.

Haplotype networks are useful when exploring intraspecific relationships among recently derived haplotypes. At the intraspecific level, many phylogenetic assumptions are violated, such as non-hierarchical relationships, extant ancestors, etc. (Posada and Crandall 2001), which can result in poorly resolved polytomies (see results; Clade A; Fig. 2.). Therefore we constructed a median-joining (M-J) network (Bandelt et al. 1999) using NETWORK v 4.5.1.0 (Fluxus Technology 2008) in an attempt to better understand relationships among haplotypes found in individuals identified as *O. virginianus* collected in LC/JBH, DCOR, and NWWTD. As divergence increases, networks can become convoluted so we excluded any haplotypes found exclusively in *O. hemionus* and the divergent haplotypes found in LC/JBH that fall into clade C (see results; Clade C; Fig. 2).

Microsatellite DNA data were assessed for scoring errors and null alleles in MICROCHECKER v2.2.3 (van Oosterhout et al. 2004). Genetic diversity was evaluated for each species from each sampling location by calculating total number of alleles (N_A) and number of private alleles (A_{PR}) using GENALEX v6.1 (Peakall and Smouse 2006). Allelic richness (A_R) accounting for differences in sample sizes was estimated in in HP-RARE v1.1 (Kalinowski 2005) with a sample size of 44 genes per sample. Expected (H_E) and observed (H_O) heterozygosities per locus and sampling

location, and tests for violations of Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were calculated using ARLEQUIN v3.5 (Excoffier and Lischer 2010). The significance of the tests was assessed at $P = 0.05$ which was Bonferroni corrected for multiple comparisons among loci (Rice 1989).

Our first step in detecting population structure was to apply the clustering algorithm in STRUCTURE v2.2 (Pritchard et al. 2000). STRUCTURE utilizes a Bayesian Markov Chain Monte Carlo (MCMC) approach to cluster individuals based on minimized linkage disequilibrium and the highest posterior probability and is free from any *a priori* assumptions regarding physical sampling locations. We ran the admixture and independent allele frequencies models. STRUCTURE was set with a burn-in of 50,000 and a MCMC length of 350,000. STRUCTURE requires that the user define the number of genetic clusters (k) into which all of the individuals are assigned. The estimated true k is that at which the posterior probabilities plateau (Pritchard et al. 2007). One downfall of the program is the lack of consistent probability estimations between runs. Consequently, one must run each k multiple times to determine the stability of the probability estimates (Waples and Gaggiotti 2006). We ran STRUCTURE at $k = 2$ thru 9 with five replicates for each. Once clusters, and hence population structure, were defined, we calculated F -statistics estimators (Weir and Cockerham 1984) and tested for significance between clusters using ARLEQUIN v3.5. A second measure of differentiation, D_{EST} (Jost 2008), was estimated using

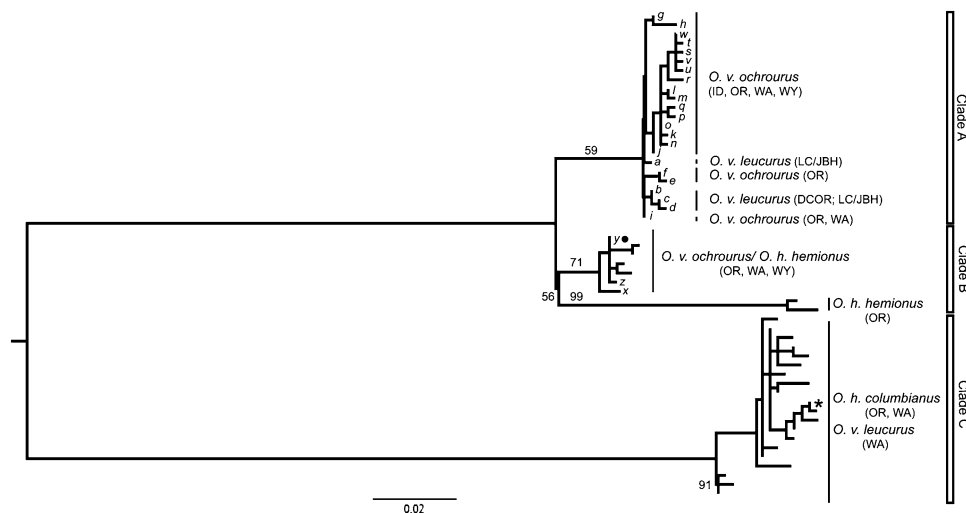


Fig. 2 Maximum likelihood phylogenetic tree generated in PAUP* for 614 base pairs of the mitochondrial DNA control region from *Odocoileus* spp. collected from the Pacific Northwest, U.S.A. The current subspecies designation and collection location (parentheses) are provided for each clade: lower Columbia River/Julia Butler Hansen Refuge (LC/JBH); Douglas County, Oregon (DCOR); Eastern Oregon (OR); NWWTD is represented by eastern Washington (WA);

Idaho (ID), and Wyoming (WY). Bootstrap branch supports >50 are presented at each node. (bullet) represents a haplotype shared by *O. v. ochrochrous* and *O. h. hemionus* and (*) represents an *O. h. columbianus* haplotype found only in *O. v. leucurus* from LC/JBHR (See Fig. 3 for exact location of this haplotype). Haplotypes from *O. virginianus* are labeled with the letter that corresponds to haplotypes in Fig. 3

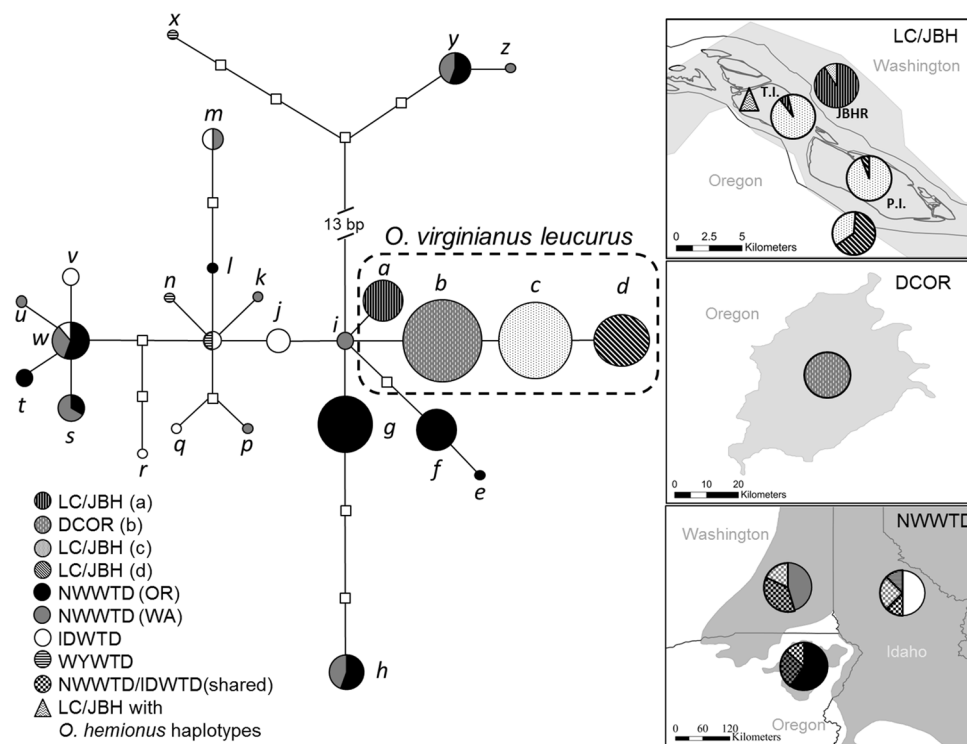


Fig. 3 Median-joining network generated in NETWORK v4.6.1 for 614 base pairs of the mitochondrial DNA control region from *Odocoileus virginianus* collected from the Pacific Northwest, U.S.A. Each circle represents a haplotype with the branch in between representing one base pair change. The size of each haplotype circle represents its frequency among all *O. virginianus* samples. The colors and patterns represent a particular sampling location and circles with two or more colors/patterns were found in multiple locations (see legend and insets). The squares represent missing/unsampled/extinct haplotypes. Haplotypes found in *O. v. leucurus* are labeled and have designated letters (a–d). The insets show the location of each haplotype: lower Columbia River/Julia Butler Hansen Refuge (LC/JBH); Douglas County, Oregon (DCOR); Eastern Oregon (OR); Eastern Washington (WA); Idaho (ID) and Wyoming (WY). The circles within the insets

demonstrate the geographical distribution of the haplotypes (see legend). The checkered pattern haplotypes in the OR, WA, ID inset represent haplotypes shared with another location within the inset. For example a grey/white checkered pattern means those haplotypes are shared among the locations marked with solid grey and solid white (see legend). A solid color in the OR, WA, ID inset means that those haplotypes were only found in that location. The triangle in the LC/JBH inset represents the collection location of the *O. v. leucurus* individuals with the *O. h. columbianus* haplotype (see Fig. 2). The abbreviations in the LC/JBH inset represent: Julia Butler Hansen Refuge Washington mainland (JBH); Puget Island, WA (P.I.) and Tenasillahe Island, OR (T.I.). Letters at nodes are haplotype designations and correspond to those in Table A3 (electronic supplementary material)

SMOGLD (Crawford 2010) as F_{ST} has been shown to asymptote as marker variance increases (Jost 2008).

Results

Mitochondrial diversity, phylogeny, and geographic distribution

Mitochondrial DNA sequences were generated for $n = 291$ individuals (LC/JBH = 80, DCOR = 44, IDWTD = 10, NEWTD = 2, NWWTD = 77, WYWTD = 3, BTD = 52, and MD = 23). We successfully sequenced 614 base-pairs, of which 88 sites were parsimony informative with 4 indels. Across all individuals, we recovered 52 haplotypes, 27 found in individuals identified as *O. virginianus* and 25 in individuals identified as *O. hemionus* (Table A3 in

electronic supplementary material). The mtDNA diversity of the 23 *O. virginianus* haplotypes (excluding haplotypes that grouped with *O. hemionus* in Fig. 2) consisted of 12 polymorphic sites, 0 indels, $h = 0.77$, $\pi = 0.0074$, and the mean number of nucleotide differences was 3.8.

A single ML tree revealed three main clades (Fig. 2). Clade A consisted of *O. virginianus* haplotypes from LC/JBH, DCOR, IDWTD, NWWTD, and WYWTD. The branch lengths within this clade were short and mean sequence divergence among haplotypes was low (0.74 %; range: 0.163–1.47 %) which resulted in unresolved polytomies and bootstrap values less than 50 %. Clade B consisted of haplotypes found in *O. h. hemionus* and *O. virginianus* from NWWTD and WYWTD and the mean sequence divergence within was 2.21 % (range: 0.163–4.89). This clade was further divided into two sister clades with much higher branch support. One clade consisted of both *O.*

Table 1 Genetic diversity estimates from 16 autosomal microsatellite loci for 276 Pacific Northwest *Odocoileus* spp.

Locus	LC/JBH (n = 80)			DCOR (n = 44)			NWWTD (n = 74)			<i>O. h. columbianus</i> (n = 53)			<i>O. h. hemionus</i> (n = 25)															
	A	H _E	H _O	A	H _E	H _O	A	H _E	H _O	A	H _E	H _O	A	H _E	H _O	A	H _E	H _O										
BL25	9	0.33	0.32	3	2.8	0.2	0.2	–	2	2.0	0	0	–	4	3.5	0.3	0.3	1	7	5.4	0.5	0.5	1	4	4.0	0.7	0.6	–
BM4208	11	0.71	0.61	4	4.0	0.7	0.6	1	4	4.0	0.7	0.7	–	9	8.3	0.8	0.8	3	4	4.0	0.7	0.5	–	6	5.9	0.7	0.5	–
BM6348	14	0.66	0.62	5	4.4	0.5	0.4	–	5	5.0	0.7	0.7	–	11	10.4	0.8	0.8	5	7	6.7	0.8	0.8	1	4	4.0	0.5	0.4	–
BM6506	11	0.67	0.60	5	4.8	0.6	0.4	–	4	4.0	0.5	0.4	–	11	10.1	0.8	0.7	3	6	5.4	0.8	0.7	–	5	5.0	0.7	0.8	–
BM848	11	0.64	0.54	4	3.5	0.6	0.5	–	3	3.0	0.7	0.5	–	7	6.6	0.4	0.2	3	8	7.1	0.8	0.7	–	7	6.9	0.8	0.8	–
BOVPR1	2	0.22	0.20	2	2.0	0.5	0.5	–	2	2.0	0	0	–	2	2.0	0.5	0.4	–	1	1.0	–	–	–	2	2.0	0.1	0	–
Cervid_1	13	0.53	0.56	4	3.8	0.2	0.2	–	3	3.0	0.3	0.3	–	10	9.4	0.7	0.7	1	11	9.9	0.9	0.8	1	6	5.9	0.7	0.8	–
D	8	0.63	0.53	4	3.8	0.5	0.4	–	4	4.0	0.7	0.7	–	6	5.9	0.7	0.5	–	8	7.4	0.8	0.7	–	4	4.0	0.5	0.4	–
ILSTS011	10	0.65	0.52	7	6.5	0.6	0.3	–	4	4.0	0.6	0.5	–	6	5.2	0.7	0.7	–	10	8.2	0.8	0.8	2	5	5.0	0.6	0.3	–
INRA011	5	0.10	0.10	2	2.0	0.1	0.1	–	2	2.0	0	0	–	4	3.9	0.2	0.2	2	1	1.0	–	–	–	2	2.0	0.2	0.2	1
K	6	0.37	0.40	3	2.9	0.1	0.1	–	3	3.0	0.5	0.5	1	2	1.8	0	0	–	4	4.0	0.6	0.6	–	5	4.9	0.7	0.8	–
N	13	0.73	0.75	7	5.4	0.5	0.6	–	5	5.0	0.6	0.7	–	7	6.5	0.8	0.8	–	13	11.1	0.9	0.8	–	12	11.8	0.9	0.9	–
OarFCB	10	0.40	0.38	5	4.9	0.7	0.6	–	4	4.0	0.6	0.5	–	9	8.8	0.8	0.8	4	1	1.0	–	–	–	1	1.0	–	–	–
P	7	0.56	0.52	4	3.8	0.4	0.4	–	3	3.0	0.4	0.4	–	4	3.9	0.5	0.5	–	7	6.8	0.8	0.7	1	5	4.9	0.7	0.6	–
Q*	13	0.66	0.49	7	5.5	0.4	0.3	–	5	5.0	0.5	0.4	–	9	8.2	0.8	0.7	–	11	10.3	0.9	0.6	–	8	8.0	0.8	0.5	–
R	8	0.41	0.34	3	2.5	0.3	0.3	–	1	1.0	–	–	–	2	2.0	0.5	0.5	–	7	5.8	0.7	0.4	2	5	5.0	0.7	0.6	–
Mean	9	0.52	0.47	4.3	3.9	0.4	0.4	1.0	3.4	3.4	0.5	0.4	1.0	6.4	6.0	0.6	0.5	2.8	6.6	5.9	0.7	0.7	1.3	5.1	5.0	0.6	0.5	1.0

Presented in the table for each locus are the mean values across all samples and for each sampling location and/or subspecies. The sampling locations for *Odocoileus virginianus* correspond to: lower Columbia River [*O. v. leucurus* (LC/JBH)]; Douglas County, Oregon [*O. v. leucurus* (DCOR)]; Eastern Oregon and Washington [*O. v. ochrourus* (NWWTD)]; OR and WA in Figs. 1, 2, 3]. Idaho samples were not genotyped due to small sample size. The mean estimates across all loci are provided in the last row. The acronyms stand for: total number of alleles (A), allelic richness (A_R), expected (H_E) and observed (H_O) heterozygosities and total number of private alleles (A_{PR})

* Locus Q was removed from clustering analysis and genetic divergence estimates due to significant deviations from HWE in all samples

h. hemionus and *O. virginianus* from NWWTD and WYWTD. One haplotype included in this clade was shared among NWWTD ($n = 7$) and *O. h. hemionus* ($n = 4$) individuals (Fig. 2). The second clade consisted of two haplotypes from *O. h. hemionus*. Clade C included all *O. h. columbianus* and individuals identified as *O. v. leucurus* from Tenasillahe Island in LC/JBH ($n = 8$). The mean divergence within clade C was 2.16 % (range: 0.164–2.30). The sequence divergence between clades was as follows: clade A–clade B = 3.64 %; clade A–clade C = 9.60 %; and clade B–clade C = 9.65 %.

The M-J network highlights the high similarity among the Clade A haplotypes ($n = 23$) and three haplotypes (x, y, z) from Clade B in Fig. 2 (Fig. 3). The base pair differences among these haplotypes ranged from one to 19 with no haplotypes shared among NWWTD, LCWTD, and DCOR. Only four haplotypes were recovered from LC/JBH and DCOR. Three haplotypes were found in LC/JBH (a, c, d , Fig. 3). All individuals from DCOR had a single haplotype (b , Fig. 3). The base pair differences among haplotypes a – d ranged from one to four. Interestingly one haplotype (i) found in two NWWTD individuals (Fig. 3) was only one base pair different from LC/JBH haplotype (a) and the DCOR haplotype (b). No haplotypes found in LC/JBH were isolated on an island or either side of the Columbia River, but there were haplotype frequency differences demonstrating mtDNA gene flow among the islands, but not complete admixture. On the Washington mainland in LC/JBH, haplotype a had a high frequency (10 individuals; 91 %) while haplotype c occurred in just one individual (9 %). The dominant haplotype on Puget (16 individuals; 94 %) and Tenasillahe (14 individuals; 93 %) Islands was c . The two islands differed in low frequency haplotypes with a present on Tenasillahe Island (one individual; 7 %) and d on Puget Island (one individual; 6 %). Haplotype d was dominant on mainland Oregon (18 individuals; 64 %) and c was present at a lower frequency (10 individuals; 36 %).

Microsatellite loci

All microsatellite loci were highly polymorphic averaging 9.1 alleles per locus (range: 2–18; Table 1). Analyses in MICROCHECKER revealed potential null alleles in three out of four populations and potential scoring errors in one population. These significant tests were associated with nine loci, but only locus O was significant in multiple (3/4) populations. Significant deviations from HWE occurred in 6 % of the probability tests (4 of 68), with 3.4 expected by chance alone at the 5 % level (Bonferroni correction $\alpha = 0.00294$). None of the exact HWE tests for heterozygote excess were significant. However, four of 68 (6 %) of the tests for heterozygote deficit were significant.

These violations were attributed to loci O and Q which were removed from final clustering and divergence analyses (no summary statistics are available for O because it was removed from all downstream analyses). Significant LD tests occurred in 0.55 % of the comparisons (3 of 544), with 27.2 expected by chance at the 0.05 level.

Microsatellite diversity

The numbers of individuals that were successfully genotyped for each sampling location are listed in Table 1. Mean genetic diversity estimates in Table 1 were based on 16 loci. Genetic diversity varied across all species and populations with mean number of alleles ranging from 3.4 to 6.6, A_R from 3.4 to 6.0, and mean H_O and H_E from 0.4 to 0.7 per sampling location (Table 1). Both LC/JBH and DCOR had lower genetic diversity compared to NWWTD which is also lower than other estimates from *O. virginianus* from western North America (Cullingham et al. 2011). Within *O. virginianus*, NWWTD had the highest number of private alleles ($A_{PR} = 22$). LC/JBH and DCOR each had one private allele. BTD and MD had eight and one private alleles, respectively.

Population structure and differentiation

STRUCTURE analysis posterior probability peaked and plateaued at $k = 4$ clusters (Fig. 4). All *O. virginianus* were assigned to one of three clusters that correlated geographically to LC/JBH (cluster 1), DCOR (cluster 2), and NWWTD (cluster 3). Two of cluster 3 individuals had 21 and 47 % of their genotypes assigned to the cluster 1 and/or cluster 2. Two of cluster 1 individuals had 10 and 23 % of their genotype assigned to cluster 3. Two different individuals from cluster 1 were assigned 10 and 29 % to cluster 2. One individual from cluster 2 had 31 % of its genotype assigned to cluster 3. Cluster 4 consisted of all *O. hemionus* individuals. Using the four clusters identified in STRUCTURE, we estimated F_{ST} (Table 2). As expected, the largest amount of differentiation was between *O. virginianus* and *O. hemionus* ($F_{ST} = 0.40$ – 0.49). The most differentiated *O. virginianus* populations were DCOR and LC/JBH ($F_{ST} = 0.31$). NWWTD was nearly equally differentiated from both DCOR and LC/JBH ($F_{ST} = 0.17$ and 0.19 respectively). D_{EST} ranged from 0.14 to 0.19 among the *O. virginianus* populations. The highest value (0.19) was between DCOR and LC/JBH.

Discussion

In this study we analyzed mtDNA and microsatellite loci to inform translocation and recovery plans of *O. v. leucurus*.

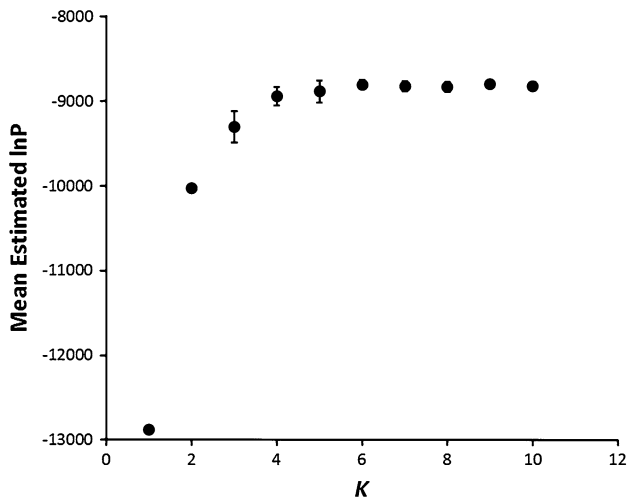


Fig. 4 Plot of mean estimated lnP (posterior probability of the data) versus the number of genetic clusters (*K*) as determined in STRUCTURE v2.3.1 for 276 Pacific Northwest, USA *Odocoileus* spp. genotyped at 15 autosomal microsatellite loci. The associated standard deviations are represented by vertical bars at each data point

The four major findings of this study were: (1) low mtDNA divergences that did not validate/support subspecific designations of white-tailed deer; (2) discovery of a contact zone between two divergent lineages of *O. virginianus* in northeastern Oregon based on co-occurring divergent haplotypes; (3) little or no contemporary gene flow among *O. virginianus* populations based on lack of shared haplotypes and high F_{ST} , and lower genetic diversity west of the Cascades; and (4) historical introgression of *O. h. columbianus* mtDNA haplotypes into the endangered population of *O. v. leucurus* from the Lower Columbia River.

Phylogeny, genetic diversity, and gene flow

The phylogenetic data presented herein suggests *O. virginianus* in the PNW are descendents of a recent evolutionary origin from a single panmictic population. We found extremely low mtDNA divergences (mean $\leq 1\%$), unresolved phylogenetic relationships among haplotypes in Clade A, and paraphyly of the NWWTD haplotypes in relation to LC/JBH and DCOR, which suggested little congruence with current taxonomy (Fig. 2). The network analysis further corroborated the phylogenetic tree by identifying four haplotypes (*a, b, c, d*) in DCOR and LC/JBH samples that were only one to three base pairs different from haplotype *i* which is found in two individuals from NWWTD (Fig. 3). Yet despite the genetic similarity, no haplotypes were shared among the three main sampling locations (DCOR, LC/JBH, NWWTD). The lack of shared haplotypes could be a function of biased sampling, but considering our sample sizes, the most logical scenario is

Table 2 Matrices of two genetic divergence estimates for 15 autosomal microsatellite loci amplified from *Odocoileus* spp. from the Pacific Northwest, U.S.A.

	LC/JBH	DCOR	NWWTD	BT	MD
LC/JBH	–	0.19	0.14	0.73	0.76
DCOR	0.31	–	0.15	0.75	0.82
NWWTD	0.19	0.17	–	0.77	0.78
BT	0.46	0.47	0.40	–	0.14
MD	0.49	0.49	0.41	0.10	–

The upper matrix is D_{EST} (Jost 2008) and the lower matrix is F_{ST} . The location acronyms are: lower Columbia River *O. virginianus leucurus* (LC/JBH); Douglas County, Oregon *O. v. leucurus* (DCOR); Eastern Oregon and Washington *O. v. ochrourus* (NWWTD); *O. hemionus columbianus* (BT black-tailed) and *O. h. hemionus* (MD mule deer). Idaho, Wyoming, and Nebraska samples were not included in these estimates due to low sample size

that *O. virginianus* on both sides of the Cascades were historically connected and widespread; Euro-American settlement then fragmented the deer into the three contemporary populations. Closely related haplotypes were sorted among the disjunct geographic locations by genetic drift. Although our results suggest that LC/JBH and DCOR may not be a monophyletic subspecies distinct from deer in NWWTD (based on the phylogenetic species concept), we cannot determine whether all should be considered *O. v. leucurus* or *O. v. ochrourus* because we do not have samples from the broader distribution of *O. v. ochrourus*, and from other sympatric and parapatric subspecies. Further, Cronin (1992) found that most of the putative subspecies shared mtDNA RFLP haplotypes thus the total genetic data do not support the current subspecies designations.

The microsatellite and mitochondrial diversities of the NWWTD population were higher than both LC/JBH and DCOR (Table 1). Further, the LC/JBH population had a higher mtDNA diversity than DCOR despite the significantly larger population size of the latter (349 vs. 6,000; Federal Register 50 FR43647; P. Meyers unpublished data). The lower genetic diversity and relationships of the LC/JBH and DCOR populations can be explained by several possible scenarios. White-tailed deer from NWWTD may have formerly ranged in the entire eastern portion of Oregon and gave rise to a population of deer in the Umpqua and Willamette River Valleys. A secondary colonization could have followed the northern side of the Columbia River. Thus, the low genetic diversity in DCOR and LC/JBH is due to two founder events. Alternately, white-tailed deer may have ranged throughout Oregon and due to either climatic fluctuations, and/or anthropogenic influences, deer in the Umpqua and Willamette River Valleys and Columbia River basin were subsequently isolated and have lost the shared genetic diversity from the

more broadly distributed white-tailed deer now found in NWWTD. In this scenario DCOR suffered a greater bottleneck effect than LC/JBH based on current genetic diversity. Regardless, this is further evidence that all three populations were a single contiguous gene pool in recent history, but are currently isolated and have lost a large proportion of shared genetic diversity.

Smith et al. (2003) evaluated the cranial morphology of all three populations analyzed in this study. They found that each population was significantly distinct at multiple morphometric variables. The authors concluded that all three locations originated from a single panmictic population but the morphological evidence demonstrated contemporary fragmentation, results supported by our data. Smith et al. (2003) further interpreted their morphological data as evidence for incipient speciation. Thus, they suggested that current taxonomic designations should remain and no attempts to supplement or translocate populations should be undertaken. We respectfully disagree with this conclusion as cervids display a significant amount of phenotypic plasticity (Putman and Flueck 2011). Further, the Smith et al. (2003) study did not specifically address the influence of genetic drift on morphology, or differences in environmental conditions, such as food availability, animal density, and competition for resources, which may have influenced the different cranial sizes. Moreover, the use of morphometric data in a phylogenetic context has been questioned due to the lack of discrete diagnosable traits (synapomorphies) and tests for homology, thus the macroevolutionary basis for their conclusions is debatable (Klingenberg and Gidaszewski 2010; Pimentel and Riggins 1987). Finally, potential hazards to bottlenecked populations such as inbreeding and genetic load were not considered when they suggested restricting translocations.

Hybridization and introgression

Our data revealed introgression of a single *O. h. columbianus* haplotype in 8 of 23 (35 %) *O. virginianus* from Tenahsille Island in the LC/JBH population (Clade C; Fig. 2). As of 2010, the number of deer on Tenahsille Island was estimated at 148 (P. Meyers, unpublished data). Based on our sampling, the number of introgressed individuals on this island represents at least 5.4 % of the estimated population size. Gavin and May (1988) estimated 18.2 % of their LC/JBH samples from both the Washington mainland and Puget Island were introgressed with *O. h. columbianus* allozyme alleles. Cronin (1991) found two deer in the DCOR population with an *O. h. columbianus* mtDNA RFLP haplotype. Neither Gavin and May (1988) nor this study detected introgression in the DCOR population. The reasons for this discrepancy could be that we only sampled 44 DCOR individuals out of an estimated

population size of greater than 6,000 (Federal Register 50 FR43647) and could have failed to detect an extremely rare occurrence of *O. h. columbianus* introgression. At that time, Cronin (1991) only sampled 12 individuals but the DCOR population has since grown to double its size which could effectively decrease the detection probability of a rare haplotype. A second explanation could be that that haplotype has gone extinct since the Cronin (1991) study. Our microsatellite data did not reveal any recent hybrids (e.g., F1 or F2) in any of the sampling locations thus suggesting that hybridization was a historic occurrence. The hybridization events discovered here suggest historic breeding of an *O. virginianus* male with an *O. hemionus* female. This is intriguing because previous reports of introgression between these two species has found *O. virginianus* mtDNA in *O. hemionus* individuals (Carr et al. 1986). But later studies suggested interspecies gene flow is not unidirectional (Ballinger et al. 1992) and that *O. virginianus* males mating with *O. hemionus* females is more likely (Cathey et al. 1998).

The level of introgression on Tenahsille Island has two possible explanations. An Allee effect, due to the small, geographically isolated habitat, could have led individuals to be less selective in choosing mates, even if it was a different species (Allee et al. 1949; Lodé et al. 2005). The introgression could also have been a result of an immigration or translocation of an introgressed female/s to the island. Before translocation efforts to Tenahsille Island began in the 1980s, it was estimated that only 40 *O. virginianus* occupied the island (P. Meyers, unpublished data). As the number of deer on the island was quite small, mating success would be higher for any particular female thus increasing its contribution to the native gene pool. Because hybridization has potential implications for fitness (Lingle 1993) and fertility (Derr et al. 1991) the inability to identify these hybrids in the field poses problems for management as hybrids can be inadvertently selected for translocations. This could then result in poorly adapted populations which could ultimately lead to translocation failure.

We also found *O. h. hemionus* and *O. virginianus* mtDNA haplotypes from northeastern Oregon clustered into a single clade (Fig. 2; Clade B). One of these haplotypes was shared among *O. virginianus* individuals from NWWTD and sympatric *O. h. hemionus* individuals. This lack of mtDNA divergence among *Odocoileus* species is a well-documented occurrence with less divergence between *O. h. hemionus* and *O. virginianus* than between *O. hemionus* subspecies (Carr et al., 1986; Cronin et al., 1988). The widespread distribution of limited divergence and shared haplotypes among *O. h. hemionus* and *O. virginianus* has been interpreted two ways: historic introgression (Carr et al. 1986), and incomplete lineage sorting (Cronin et al. 1988), but resolving this is beyond the scope of this study.

Management and conservation implications

The lexicon used for intraspecific groups in conservation and taxonomy has a tortuous history with little consensus on the definitions (Cronin 2006). In fact, the role of subspecies in conservation has been an area of contention and confusion (Cronin 2006; Haig et al. 2006; Zink 2004). The original definition of a subspecies is a “geographically defined aggregate of local populations which differ taxonomically from other subdivisions of the species” (Mayr 1963). Avise and Ball (1990) provided a standardized genetic definition of subspecies as groups that are phylogenetically distinguishable from other groups at multiple genetic traits, but may still interbreed. O’Brien and Mayr (1991) formalized an applicable definition of a subspecies as sharing geographic range or habitat, multiple phylogenetically concordant characters, and a unique natural history when compared to other intraspecific subdivisions. Some authors have consequently pointed out though that many subspecies have been defined based on single qualitative trait (e.g., size or color) and when reevaluated, additional characters were interpreted through the lens of the original taxonomic designation (Wilson and Brown 1953).

The data presented herein has identified very little phylogenetic divergence, but confirmed contemporary geographic isolation. Considering that we only used a single phylogenetically informative marker (mtDNA), and that genetic and morphometric data disagree (Cronin 1992; Gavin and May 1988; Smith et al. 2003) we cannot make a final taxonomic conclusion about the subspecific status of *O. virginianus* populations in Oregon and Washington. However, multiple sets of genetic data, this study, Gavin and May (1988), Cronin (1991), and Cronin (1992), have not supported the validity of the subspecies in the PNW. Certainly based on the definitions of intraspecific units by Moritz (1994) each population should be a distinct management unit due to genetic differentiation and lack of gene flow at nuclear microsatellite loci but no reciprocal monophyly of mtDNA. Considering that anthropogenic effects most likely caused the contemporary differentiation, ecological and genetic connectivity should be restored to the historical condition (Crandall et al. 2000). In the case of *O. virginianus* in the PNW, which has conservation value in its own right, the best method to do this is through translocations. The peripheral nature and relictual status of these populations validates the conservation value as these populations most likely experienced different selective pressures than populations in the more densely populated portions of their range (Lesica and Allendorf 1995). Thus, each population may harbor unique adaptations and genetic variation which could be important for persistence of the species under changing environmental conditions (Sgrò et al. 2011) confirming that conservation and expansion of

the native *O. virginianus* populations are legitimate management objectives.

The recent panmictic origin of LC/JBH and DCOR supports the use of these populations to supplement each other, and to reestablish populations within their historic range throughout western Oregon and Washington. Increasing the genetic diversity in both populations (DCOR and LC/JBH) could lead to enhanced population resilience and adaptability to changing environments (Hedrick et al. 2001; Sgrò et al. 2011; Spielman et al. 2004). Establishing new populations would further reduce the extinction probability of *O. v. leucurus* due to a decreased likelihood that a stochastic event would wipe out one or both of the current populations. One area of caution though is the use of a single inbred population as a source for reintroductions. Reintroductions can cause additional genetic bottlenecks which can lead to further reduction in genetic diversity and survival, thus wasting time, effort, and animal lives (Jon and Witham 1990; Sgrò et al. 2011). The best initial strategy for reintroductions would be to use both LC/JBH and DCOR as source populations. Transfer of *O. virginianus* from NWWTD to west of the Cascades is also feasible, however, it must be considered carefully. The co-occurrence of a divergent *O. virginianus* clade, evidence of potential introgression with *O. h. hemionus* mtDNA, and significant differences in animal size could cause problems such as maladaptation and dystocia (difficult parturition due to breeding of animals of different proportions) (Galindo-Leal and Weber 1994). Yet, this population could be viewed as a significant source of genetic diversity to be introduced into LC/JBH and DCOR and with careful assessment and planning; a successful translocation program from this area could be established. A prime example of successful translocations using distant subspecies is the case of the Florida panther (*Puma concolor coryi* Kerr) where introduction of *P. concolor stanleyana* Kerr from Texas increased individual fitness within 4–5 generations (Hedrick and Fredrickson 2010).

Hybridization is also a significant conservation concern because it can dilute the gene pool of T and E species and lead to genetic extinction (Rhymer and Simberloff 1996). To prevent the spread of introgression when reintroducing populations, individuals from LC/JBH and DCOR should be tested by a genetic laboratory before translocation to determine potential hybrid ancestry. The other option would be to perform a quantitative study of the morphology of the introgressed individuals in an attempt to identify any visual characteristics that could lead to positive field identification.

We conclude that the subspecific taxonomic status of *O. v. leucurus* is not supported by the genetic data; however, the isolated and morphologically distinct populations that are found west of the Cascades warrant protection from further loss of genetic diversity and population numbers. In

light of this, translocation strategies between the populations west of the Cascades and even between them and the population east of the Cascades are valid approaches for increasing this genetic diversity and facilitating population recovery. Because we do not fully understand the genetic correlates for long-term population persistence, maximizing the available genetic diversity would provide the best chances for success in rapidly changing ecosystems.

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