Piaggio AJ, Heffelfinger JR, Meyers PM, Hopken MW. 2024. Creating an endangered subspecies: a comment on Smith et al. (2024). Northwest Science 97(4): *in press*.

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Creating an Endangered Subspecies: A Comment on Smith et al. (2024)

Running footer: COMMENT ON SMITH ET AL. (2024)

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Smith et al. (2024) create a new subspecies of white-tailed deer (*Odocoileus virginianus*) occupying Douglas County, Oregon. The authors should be commended for assembling all available information on the Columbian white-tailed deer (*O. v. leucurus*) to support their argument, unfortunately the authors interpretation of the data is not compelling nor entirely accurate. Their justification for subspecific distinction is based on: 1) a misinterpretation or exclusion of relevant genetic analyses (Hopken et al. 2015; Piaggio et al. 2016; Piaggio and Taylor 2022), 2) an untenable definition of subspecies based on those genetic analyses and on clinal skull morphology of three small, isolated, and fragmented sampling localities.

Genetic Data

Mitochondrial Sequence Data

Genetic data and historical records tell us subpopulations of Columbian white-tailed deer, (*O. v. leucurus*) share a very recent common ancestor and that the anthropogenic actions resulted in population isolation and interrupted gene flow in recent history, likely within the last 100 years (Hopken et al. 2015, Federal Register 81 FR71386 2016-24790). Initial genetic analyses were based on allozymes across 35 genomic loci (Gavin and May 1988), no significant differentiation was detected between deer from Douglas County, Oregon (DCOR) and Lower Columbia River (LC)/Julia Butler Hansen National Wildlife Refuge (JBH). Later mitochondrial DNA (mtDNA) haplotypes from the rapidly evolving hypervariable Region I of the control region (HVI: 614 base pairs) were obtained from samples collected from the LC/JBH, DCOR, northeastern OR, southeastern WA, ID, and WY. These largely grouped in a single Clade A (Hopken et al. 2015, figure 2) with a mean sequence divergence of 0.74%. Relationships of the 23 haplotypes within this clade are unresolved as there is very little genetic diversity or genetic distance among haplotypes (range 0.163-1.47% sequence divergence). Hopken et al. (2015) recovered only four haplotypes from individuals sampled at LC/JBH (except one that fell into a Columbian black-tailed deer [*O. hemionus columbianus*] clade, denoting introgression between these species at JBH) and DCOR (LC/JBH = 3, DCOR = 1). Hopken et al. (2015, figure 3) was not used in Smith et al. 2024 but is included here (Figure 1). This figure demonstrates that these four *O. v. leucurus* haplotypes are 1-4 base pairs different from one another with haplotype *c* from LC/JBH being only 1 bp different from haplotype *b* in DCOR, but 3 bp different from another haplotype (*a*) in LC/JBH. Further, haplotype *a* from LC/JBH is 2 bp different from the DCOR haplotype (*b*). Remarkably, there is a haplotype of the Northwest white-tailed deer (NWWTD, *O. v. ochrourus*) that is a single bp different from both *a* (LC/JBH) and *b* (DCOR) haplotypes. Earlier work by Cronin (1991) was the first to identify a shared mtDNA haplotype between DCOR, LC/JBH, and NWWTD (haplotype c; Cronin 1991). These data clearly illustrate the recent shared ancestry among these haplotypes representing two subspecies (*O. v. ochrourus* and *O. v. leucurus*) including both subpopulations of *O. v. leucurus*. Smith et al. (2024) ignored the fact that the DCOR haplotype they sampled is intermediate among the three haplotypes at LC/JBH and 1 bp different from LC/JBH and NWWTD (Figure 1).

Smith et al. (2024) based the subspecific distinctiveness on the fact that haplotypes detected in each *O. v. leucurus* subpopulations were not shared. This ignores clear shared ancestry and effects of the process of random mutation and genetic drift in a small population that likely led to the single base change between the haplotypes of *O. v. leucurus* subpopulations and between them and *O. v. ochrourus*. The HVI is often used for infraspecific analyses specifically because of its high mutation rate and phylogenetic resolution (Hasegawa et al. 1993; Wakeley 1993).

Separating the DCOR population from LC/JBH and those from *O. v. ochrourus* taxonomically creates a paraphyletic relationship among haplotypes that have a mean sequence divergence of ≤1% (Hopken et al. 2015).

We analyzed 36 additional samples collected from LC/JBH during 2016-2021 (Piaggio and Taylor 2022). All but six matched two of the three haplotypes from Hopken et al. (2015) already found in LC/JBH (GenBank Accession # KP308222.1 from Cathlamet, WA, and GenBank Accession # KP308266.1 from Westport, OR). The other 6 individuals had mitochondrial DNA haplotypes that were identical to two haplotypes (GenBank Accession # KP308229.1 and GenBank Accession # KP308236.1) both from Tenasillahe Island (Piaggio and Taylor 2022), that are more closely related to *O. h. columbianus* than to any white-tailed deer samples (Hopken et al. 2015). This apparent introgression of *O. h. columbianus* into *O. v. leucurus* was previously described as an ongoing threat to the genetic diversity of the LC/JBH population which could be exacerbated by isolation from other shared ancestral gene pools (Gavin and May 1988, Cronin 1991, Hopken et al. 2015, Piaggio and Taylor 2022). Smith et al. (2024) do not address the potential for these hybrids to be included in their analyses and influence the morphological characteristics they analyze (see below under **Cranial Morphology**).

Microsatellites

Piaggio et al. (2016) identified 13 new alleles from LC/JBH in 101 samples collected in 2014 and 2015. These samples came from *O. v. leucurus* that were being translocated from LC/JBH to the Ridgefield National Wildlife Refuge (RNWR) and Cottonwood Island, Wahkiakum County, Washington. The USFWS had been translocating Columbia River *O. v. leucurus* among islands of the LC/JBH and from both the Oregon and Washington mainland since 1984 to facilitate gene flow (Azerrad 2016) and to increase the number of subpopulations. Of the 13 new alleles identified in the LC/JBH samples, 9 were also seen in either DCOR or *O. v. ochrourus*. One of the new shared alleles between LC/JBH and DCOR was previously documented as a private allele (Hopken et al. 2015) and was used by Smith et al. (2024) to argue for taxonomic distinctiveness for the DCOR subpopulation. However, in a larger analysis (Piaggio & Taylor 2022) across 409 samples, allele 192 at locus K, had a frequency of 0.28 in DCOR and 0.007 in LC/JBH (specifically in the mainland WA population), illustrating the difficulty of relying on rare private alleles in small subpopulations as the basis for taxonomic revision. Further, two individuals captured at LC/JBH clustered with the DCOR population, meaning they shared more allelic diversity with DCOR samples than with LC/JBH (Piaggio et al. 2016). This result is not surprising given that in 2010, eight individuals were moved from DCOR to LC/JBH (Azerrad 2016) and seven of these were genotyped and included in the analysis (Piaggio et al. 2016). It is also predictable that Piaggio et al. (2016) found new alleles in LC/JBH in the 101 new samples in addition to the 80 samples analyzed by Hopken et al. (2015) because greater sample size increases the chances of detecting rare alleles. Overall, the subpopulations of *O. v. leucurus* do not meet the subspecies definition of Smith et al. (page XX), given they have more shared alleles between them than private ones that separate them. Given the logic in Smith et al. (2024) each population with a private allele at a neutral locus would be candidate for subspecies designation.

Piaggio and Taylor (2022) further analyzed 409 *O. v. leucurus* individuals and found allele 159 at locus BM4208 still a private allele for LC/JBH subpopulation of *O. v. leucurus*, and it was also found across all 5 sampling localities at LC/JBH. Smith et al. (2024) rely on a STRUCTURE

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plot (Figure 4; Figure 6, Hopken and Piaggio 2009; text Hopken et al. 2015) to argue that the LC/JBH and DCOR populations are distinct. However, they state in the text, which is verbatim from Hopken et al. 2015, that there are individuals with shared assignment between LC/JBH and DCOR. More importantly, STRUCTURE plots can appear to show clear differentiation in populations with low genetic diversity because they have different allele frequencies. Thus, further data and interpretation with an understanding of evolutionary processes (such as genetic drift in this case) are required to assess connectivity rather than simply relying on a visual plot (Lawson et al. 2018). Further, there were two genetic clusters within LC/JBH using STRUCTURE (Piaggio and Taylor 2022, figure 2), rather than the single one identified previously (Hopken and Piaggio 2009; Hopken et al. 2015). It is clear, that these two genetic clusters are not isolated breeding populations, but in fact share some gene flow given geographical proximity and known translocation history (Piaggio and Taylor 2022, figure 1). However, given Smith et al.'s subspecific designation of the DCOR subpopulation based on a STRUCTURE plot, these too could be considered separate subspecies.

*F*st

*F*st is a metric that describes the reduction of heterozygosity due to genetic drift and thus can identify population subdivision (Hartl 1981). It is used to estimate relative differences between subpopulations but should not be used as a basis for taxonomic revision. In fact, low overall genetic diversity within a population can lead to inflated *F*st between some genomic regions. If one population has a certain allele, or set of alleles, and another population has a different allele, this does not mean that there is no gene flow or that other regions do not show lower *F*st (Cruickshank and Hahn 2014). Smith et al. (2024) lean heavily on an oversimplified

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interpretation of *F*st to describe their new infraspecific designation. *F*st can be inflated by low genetic diversity (Jost 2008) due to genetic drift and inbreeding. Further, *F*st between subpopulations of *O. v. leucurus* may be detecting historical isolation-by-distance, which is a common pattern of population structure in white-tailed deer (Cullingham et al. 2011, Bauder et al. 2021, Burbrink et al. 2022). As such, these subpopulation differences are not valid support for a new taxonomic designation for one of the subpopulations.

The genetic basis for creating a new subspecies consisting of the DCOR subpopulation of *O. v. leucurus* as argued by Smith et al. (2024) comes down to the single private allele across the LC/JBH subpopulation, *F*st values, and a single haplotype that is found in only the DCOR population but is 1 bp different from LC/JBH and northeastern Oregon populations. The alternative and most parsimonious explanation of these patterns is random genetic drift in two subpopulations that were part of larger, historical population but have been isolated by habitat fragmentation resulting in lower genetic diversity and inbreeding.

Cranial Morphology

Smith et al. (2024) identified statistically significant morphological differences between *O. v. leucurus* and *O. virginianus* from northern Idaho. These differences are primarily related to overall size of the skulls (basilar length, least interorbital breadth, zygomatic breadth, and mastoid breadth), which are subject to strong environmental influences. Smith et al. (2024, figures 1 and 2) show a general body size cline from larger deer in northern Idaho (Bonner County *n* = 8 and Latah County *n* = 1), west into Wahkiakum County, Washington (*n* = 24) and Tenasillahe Island, northern Oregon (*n* = 1), and south into Douglas County in southern Oregon

(DCOR, *n* = 63) being the smallest. This sampling scheme is noteworthy given that Bonner County, Idaho, is approximately 500 miles from the next sampling locality, Wahkiakum County, Washington, and about 650 miles from the subpopulation in question in Douglas County, Oregon. Nonetheless, considering there is a well-established clinal size relationship in deer (Heffelfinger and Heffelfinger 2023) it should not be surprising that three small and isolated populations along that cline would show statistically significant differences. It is questionable, however, whether these represent taxonomically relevant differences or simply a difference in nutritional resources available. They also claim that habitat differences between LC/JBH and DCOR "…have imposed selective pressures", however, this is pure speculation as they assume that skull size is completely due to selection but no test for selection or heritability was attempted for these populations.

Smith et al. (2024, figure 3) describe the results of their principal component analysis as representing "*slightly overlapping groups*", however there is a considerable amount of overlap, likely owing to the recent gene flow of these populations before anthropogenic fragmentation of their habitat. Smith et al. (2024, figure 2B) shows individuals from the LC region overlapping most of the samples from other 2 populations. This overlap is also counter to the ability to diagnose individuals as one of the subspecies because it is based on a test of means rather than a diagnostic trait which limits classification of a future, random individual. One quantifiable definition of subspecies is that 75% of individuals in one subspecies must fall outside 99% of the other (Amadon, 1949, Patten and Unitt 2002). While not all taxonomists accept this definition, it is an attempt to make morphometric measurements diagnosable. A cursory review of the PCA indicates that it appears to violate this 75% rule, thus there are no characters to distinctively classify most individuals as DCOR or LC/JBH. In addition, there is a danger of interpreting morphological information without considering the extent of hybridization in these samples. The LC/JBH subpopulation is known to contain individuals with *O. h. columbianus* genes, which may affect some morphological traits. Figure 2B (Smith et al. 2024) contains several outliers from the LC/JBH subpopulation. The uncertainty of the lineage of these samples adds more ambiguity to the reliability of the morphological data. Given the environmental plasticity of skulls independent of phylogenetic decent and potential for hybrids, analyses of a few small and recently isolated deer populations at this regional scale do not provide compelling support for creating a new subspecies of deer that is the *O. v. leucurus* subpopulation from DCOR.

Untenable Subspecies Concept

Taxonomic separation of *O. v. douglasi* out of what is now *O. v. leucurus* in Douglas County, Oregon is contrary to conservation community efforts to increase landscape connectivity and pools of genetic diversity of deer populations throughout the western North America (Middleton et al. 2020, Kauffman et al. 2022). The genetic data from *O. v. leucurus* subpopulations support the historical record that they were all part of a regional panmictic population. Over-splitting taxonomic categories can invite activist litigation (Baier 2015), dilute resources available to imperiled taxa, and impede conservation by discouraging translocations and other efforts to foster evolutionarily potential through restoring once shared genetic diversity (Latch and Heffelfinger 2022). Further, introgression of *O. hemionus* genome into *O. v. leucurus* is a threat to the integrity of the unique genetic diversity remaining, given that in small populations rare and/or recessive alleles can become common (Rhymer and Simberloff 1996). Ralls et al. (2018) reviewed the genetic rescue literature and outlined an approach that reduces extinction risk of isolated populations that were once part of a larger widely distributed species. They determined that populations that are "...chromosomally compatible, were isolated by human activities within the last 500 years and are not adapted to strongly different environments" should be managed in a way to increase genetic diversity rather than maintain isolation. The fact that deer from DCOR have survived and increased the genetic diversity already in the LC/JBH population counters the argument that they are now uniquely adapted to DCOR and demonstrates that the populations are "chromosomally compatible". The question, as we see it, is whether or not we should allow the separation imposed by anthropomorphic alterations to natural corridors of gene flow to continue to exist and erode genetic diversity. We argue that taxonomic distinction of the *O. v. leucurus* subpopulations is invalid and instead that evolutionary potential of white-tailed deer west of the Cascade mountains would benefit from a restored shared gene pool.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

AJP, JRH, PMM, and MWH all contributed to conceptualization and writing. AJP and MWH also did data analysis.

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Figure Legend

Figure 1. 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. (Hopken et al. 2015, figure 3). 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 or patterns were found in multiple locations (see legend and insets). The squares represent missing/unsampled/extinct haplotypes. 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). Haplotypes found in *O. v. leucurus* are labeled and have designated letters (*a*–*d*). Note that haplotype *b* from DCOR is intermediate between *a*, *c*, and *d*, all from LC/JBH. Also, that *a* and *b* are one base difference from *i*, which is a NWWTD from WA. Finally, *a* is more closely related to *i* than to other haplotypes from LC/JBH (*c* and *d*). 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/JBHR inset represents the collection location of the *O. v. leucurus* individuals with the *O. h. columbianus* haplotype (Hopken et al. 2015, fig. 2). The abbreviations in the LC/JBHR inset represent: Julia Butler Hansen NWR 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 Hopken et al. 2015).