Evaluating the Extent of Hybridization between Mule and White-tailed Deer in Western Canada

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Systematics and Evolution

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Abstract

Mule deer (*Odocoileus hemionus*) are endemic to a wide variety of habitats in western North America, many of which are sympatric with their closely related sister-species white-tailed deer (*O. virginianus*). Hybridization of mule and white-tailed deer has historically been observed on a semi-regular basis throughout this range. Close monitoring of this hybrid system continues to be a priority among contemporary evolutionary biologists because (a) hybridization is a volatile evolutionary phenomenon, with potential outcomes ranging from adaptive introgression to local extinction, (b) deer are ecologically, economically, and culturally significant species, and (c) chronic wasting disease (CWD) currently poses a threat to farmed and wild cervid populations in western Canada and northern United States. CWD is a communicable, fatal, neurodegenerative disease similar to scrapie in sheep and bovine spongiform encephalopathy in cattle. Molecular ecological studies have confirmed the presence of hybrids in various regions of species overlap, including Alberta, Montana, and Texas, but a lack of detection power has limited their identification abilities to the earliest, most admixed generations. Furthermore, female F1 hybrids can sometimes retain fertility, giving rise to the possibility of advanced-generation backcrosses, which require considerable diagnostic power to be reliably identified. In this thesis, I interrogated the Alberta region of the hybrid zone with the goals of (a) better characterizing its current state and evolutionary trajectory and (b) determining whether hybridization events provide an opportunity for CWD transmission to bridge the species gap. To answer these questions, I first developed a SNP assay using 40 species-discriminating loci to improve upon the previously available molecular methods of hybrid identification. After assessing its utility and efficacy through analysis of simulated hybrid populations, I used it to conduct a comprehensive survey of wild

deer populations in western Canada. To investigate whether hybridization facilitates interspecific CWD spread, I designed sampling strategies to compare hybridization rates between infected and uninfected deer. We observed overall hybridization rates of 0.3-1.1%, slightly lower than reported elsewhere in the greater hybrid zone. The admixed individuals are independent of geographical factors and no significant association between hybridization and CWD infection status was found. Although the two species often exist in sympatry and share similar diets and life history traits, interspecific gene flow appears to be prevented by a number of pre and postzygotic barriers to reproduction, including fine-scale habitat segregation and severe underdominance of hybrid offspring. Here, I build upon past studies of introgressive hybridization of deer in Alberta by increasing hybrid detection power, expanding sample sizes, and providing a new molecular resource applicable to future research. This comprehensive, robust, and current assessment of the circumstances of hybrids in wild populations will inform stakeholders of patterns of contemporary hybridization so they may shape future research and policies accordingly.

Preface

Chapter 2 of this thesis was published as T. Russell, C. Cullingham, A. Kommadath, A. Herbst, P. Stothard, and D. Coltman, “Development of a novel mule deer genomic assembly and species-diagnostic SNP panel for assessing introgression in mule deer, white-tailed deer, and their interspecific hybrids” *G3: Genes|Genomes|Genetics,* vol. 9, issue 3, 911-919, doi:10.1534/g3.118.200838. Since I served as primary author of the original manuscript, I’ve included the full article here as it appears in the journal. I was responsible for identifying species-discriminating loci, simulating datasets, evaluating assignment capacity, performing molecular laboratory work, and writing the manuscript. CC advised on project decisions and coordinated SNP genotyping and pure species[1] validation samples. AK performed WGS, analysis of MD genome, and mapped SNPs to red, mule, and white-tailed deer genome assemblies. PS performed WGS and analysis of mule deer genome. AH completed bench work, including DNA extraction, on mule deer individual used in WGS. DC designed hybridization project and advised on decisions. All authors contributed to the editing and writing of the manuscript. The species-diagnostic SNP panel discussed in this chapter was developed with assistance from the genomic assembly produced by AK, PS, and AH. The article is included here in its entirety for clarity and continuity purposes.

As I submit this thesis, chapter 3 is in late editing stages in preparation for journal submission. For this reason, chapter 3 appears here as it will in a hypothetical future journal article (ideally, pending any editor revisions), including its own reference section. Citations of “Russell et al. 2019” are in reference to chapter 2, which also appears in the final bibliography. Co-authors for this chapter include Margot Pybus, Mark Ball (both of Alberta Fish and Wildlife), Catherine Cullingham (of Carleton University), and David Coltman (of the University of Alberta). Co-authors contributed samples, project guidance, and manuscript edits. I served as lead author of the manuscript and was responsible for the analyses and their interpretations.

1 In this thesis, I will refer to individuals with no detectable introgression as "pure species". This is a description of their hybrid status only, independent of fitness or any other sociological biases.

Acknowledgements

I'd like to thank my co-supervisors, David Coltman and Catherine Cullingham, for their academic guidance and support and for believing in me enough to give me this opportunity to further my career in research.

Thank you to the greater Molecular Ecology lab group: Sam Deakin, Arianne Sawyer, Christie Bubac, Rhiannon Peery, Josh Miller, Corey Davis, and Anh Dao. They were an incredible network of knowledge that pushed me to achieve and supported me throughout my time here.

More generally, I appreciate the University of Alberta department of biological sciences for their support in the forms of teaching assistantships and funding opportunities. Thank you to my sources of funding and samples for making this work possible: Genome Canada, Alberta Prion Research Institute, the Systems Biology and Molecular Ecology of CWD project, Alberta Fish and Wildlife, Aaron Shafer (Trent University), and Emily Latch (University of Wisconsin-Milwaukee).

And finally, thanks to my family and friends in Victoria, Edmonton, and abroad. Their support has kept me grounded while motivating me to pursue my academic passions. I truly can't express my gratitude in words. In particular, to the Ives, Russell, Adkins, Blaskovich, Utterback, and Dreesman families: thank you!

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Chapter 1: Introduction

Hybridization

Intuitively, the first and most important requisite component of sexual reproduction is a pair of fertile parents. That they should belong to the same species is justly implicit, yet even this basic assumption is regularly broken in nature. After all, the many definitions of “species” and the designation of species as the fundamental level of biological organization were constructed by humans for the sake of conveniently describing natural biota (in this paper I will default to the ecological species concept definition, where each species occupies a distinct niche (Van Valen 1976; Andersson 1990)). Certain cases of heterospecific pairings can and do produce offspring, a phenomenon called hybridization. The vast majority of interspecific matings, however, are prevented by reproductive barriers (Abadie et al. 2011). During the speciation process, these barriers increase pre and/or postzygotic isolation between lineages (Orr and Presgraves 2000). As the newly formed clades continue on their divergent evolutionary paths, the barriers tend to increase in strength and number (Martin and Mendelson 2018). The species may continue to diverge and become completely genetically isolated to the point that they no longer share an evolutionary fate (Baker and Bradley 2006). The end result is two genetically distinct species. Alternatively, during instances of hybridization, the isolating mechanisms of one or more barriers fail and the two species are allowed to reproduce offspring. The hybrid offspring are a blended representation of their two parental species.

Strong isolating mechanisms that differentiate populations drive divergence and can potentially lead to speciation. It follows, then, that the convergence of those mechanisms may lead to hybridization. For example, many birds use songs as important signals when choosing a mate. Qvarnström et al. (2006) found that some male pied flycatchers (*Ficedula hypoleuca*) incorporated sounds from collared flycatchers (*F. albicollis*). Those birds that sang mixed songs often hybridized with collared flycatchers, whereas birds that sang strictly pied flycatcher songs did not. The species-specific songs are an effective prezygotic barrier until they converge. When a song ceases to be exclusive to one species, its ability to act as a barrier to reproduction is diminished.

Geographic and habitat isolation can restrict gene flow in a similar way. Populations that exchange very few migrants experience genetic drift and local adaptation independently, all while continuing to diverge from one another. But, like bird songs, if this barrier were to be removed, the lineages may still be genetically similar enough to hybridize. This is commonly seen among non-native species transplantations (Mooney and Cleland 2001). For example, in areas where mallard ducks (*Anas platyrhynchos*) have been introduced, they have hybridized with the New Zealand Grey duck (*A. superciliosa superciliosa*), the Hawaiian duck (*A. wyvilliana*), and the Florida mottled duck (*A. fulvigula fulvigula*), always at the cost of reducing native populations (Rhymer and Simberloff 1996). In each case, the species had evolved for a time in allopatry before reuniting and hybridizing in sympatry. Anthropogenic interference can displace non-native species directly (e.g. intentional or accidental translocation) or indirectly (e.g. habitat fragmentation). Both can create opportunities for hybridization and are increasingly common in the present age of escalating globalization and

habitat modification (Grabenstein and Taylor 2018; Mallet 2005; Rhymer and Simberloff 1996).

The repercussions of hybridization are just as varied as its causes. Natural hybridization, as a potentially creative evolutionary process, can be a source of gene flow and thus allele sharing, adaptive introgression, and hybrid vigour are all potential outcomes (Dowling and Secor 1997; Martinsen et al. 2001). By introducing genetic novelties more rapidly than spontaneous mutations alone, hybridization can increase allelic variation—which may include adaptively important traits—and even play a role in speciation (Kim et al. 2008; Hegarty and Hiscock 2008). But more often hybridization seems to be detrimental to the parental species involved, especially when caused by human disturbance (Casas et al. 2012; Hornbeck and Mahoney 2000). In fact, a meta-analysis by Todesco et al. (2016) found that studies of hybridization associated with human involvement (defined as species introduction, habitat disturbance, or husbandry) were more likely to report a threat of extinction than studies of natural hybridization. Hybridization may increase the risk of local or global extinction in one of two ways: demographic swamping or genetic swamping (Todesco et al. 2016; Balao et al. 2015; Roberts et al. 2010).

Briefly, demographic swamping describes the decline of parental populations due to the wasted reproductive effort of rearing sterile hybrids. Hybrid offspring often experience fertility issues as a symptom of outbreeding depression (also known as heterozygote breakdown and underdominance). Outbreeding depression is caused by the compromising of coadapted gene complexes and the accumulation of Dobzhansky-Muller incompatibilities, which is in turn caused by the heightened dissimilarity of the parental species coming together to form a

hybrid genome (Frankham et al. 2011). Because hybrid genomes are a composite of two divergent parental genomes, some allele combinations that have never previously existed together may be present. There is no guarantee that these will work effectively in the same individual because they evolved in separate genetic backgrounds (Orr and Turelli 2001). For example, polygenic traits may require loci from various parts of the genome working together to function properly, but if the genome is fragmented into linkage groups that evolved in different genetic backgrounds (i.e. parental species A vs. parental species B), those interspersed loci may not be compatible. Such alleles that are disruptive in a non-native genetic background were first described by Dobzhansky (1936) and Muller (1942).

Alternatively, genetic swamping can occur if outbreeding depression is less severe (Bouzat et al. 2009). Genetically swamped parental species are replaced by a hybrid swarm in which a large proportion of the population is hybridized (Coleman et al. 2014). In this scenario, the parental genomes are extirpated but their alleles survive in hybrids. Genetic swamping is likely much more frequent in nature than demographic swamping (Todesco et al. 2016).

Extinction is just one possible outcome in a diverse array of hybridization-mediated consequences. Buerkle et al. (2003) investigated the relative frequency of three of those outcomes—speciation, extinction, and maintenance of a stable hybrid zone—using simulations with a variety of ecological and genetic parameters. They found speciation to be the least frequent outcome (2.1% of simulations); it required high levels of F1 fertility and ecological selection. Extinction was rare as well (13.9% of simulations) and only occurred via genetic swamping, i.e. high F1 fertility and no habitat separation. The most common

outcome of their simulated hybrid systems, and presumably the most likely to be observed in nature, was the maintenance of a stable hybrid zone (84% of simulations). It seems the hybridizing taxa were able to coexist across all ecological selection gradients and all but the highest levels of hybrid fertility, which supports the idea that demographic swamping generally poses a very low risk of extinction (Todesco et al. 2016).

Because of its wide variety of causes and effects, hybridization also bears varied management implications. Diverse impacts make drafting and implementing policies very difficult. Any policy that expects strict adherence would have to be flexible enough to apply to all situations but still provide helpful recommendations (Allendorf et al. 2001). Given the rarity of progressive evolutionary effects and the difficulty of assessing whether a given instance of hybridization benefits or harms a population in the long term, hybridization is generally viewed as undesirable. This paradigm was reflected in the US Endangered Species Act of 1973 that used elements of the biological species concept to deem hybrids unworthy of conservation. Biologists, including father of the biological species concept Ernst Mayer, argued that a strict interpretation of the policy excluded species that had experienced high levels of gene flow (O'Brien and Mayr 1991). The Hybrid Act, as it became known, was rescinded in 1990. Contemporary biologists have suggested that policies should focus on limiting the anthropogenic factors that lead to hybridization (Allendorf et al. 2001). That means avoiding species translocations, limiting habitat fragmentation and modification, and separating hybridizing taxa in captive populations among other precautions (Russo et al. 2019).

Introgressive hybridization can be a vehicle for sharing adaptive evolutionary traits among taxa, such as migration behaviour in Rocky Mountain elk (McDevitt et al. 2009), but such adaptive novelty is rare enough in animals that wildlife managers often aim to abstain from hybridization altogether. Ideally, hybridization events should be studied individually in order to determine best management practices (Allendorf et al. 2001).

Odocoileus spp.

Mule deer (MD) (*Odocoileus hemionus*) and white-tailed deer (WT) (*O. virginianus*) are sister species of economic, cultural, and ecological significance throughout North America. In 2016 Americans spent nearly $15 billion on domestic big game hunting and Canadian expenditures exceeded $1 billion in 2014 (U.S. Fish and Wildlife Service 2016; Federal, Provincial, and Territorial governments of Canada 2014). They bear cultural significance among indigenous peoples and represent a ubiquitous, recognizable symbol of ecological health among the general population. Ecologically, deer occupy a crucial intermediate position in trophic hierarchies as primary consumers and important prey species for large predators, namely wolves, bears, coyotes, and cougars (Robinson et al. 2002; Lingle 2002; Kunkel and Mech 1994).

Mule deer are found in a variety of habitats throughout western North America, from deserts in the southwest to coastal rain forests in the north and as far east as Minnesota and Iowa (Forrester and Whittmer 2013; Patterson et al. 2003). White-tailed deer distribution spans North America coast to coast and from Mexico in the south to the northern-most Canadian territories (Hewitt 2011). MD and WT range distributions overlap considerably but, even in areas of sympatry and despite sharing similar diets, habitat segregation reduces

interspecific resource competition (Berry et al. 2019). One important factor driving habitat segregation is their stark contrast in anti-predator strategies. MD, when confronted by a coyote or other small predator, tend to form cohesive groups in order to defend themselves. Alternatively, a WT group, when threatened by the same predator, will quickly disperse in an attempt to outrun their aggressor (Lingle 2001; 2002). This distinction of strategies plays to the abilities of each species: WT have a faster top speed than any of their predators, while MD are larger and more tenacious than WT and form larger, tighter social groups (Habib et al. 2011; Lingle 2001). Also, when MD are forced to flee a confrontation, they use a highly specialized stot to cover large horizontal and vertical distances in bounds instead of the more traditional gallop used by WT (Lingle 1993). The MD stot has evolved to traverse irregular terrain while the WT gallop maximizes peak velocity. In order to optimize their escape gaits, MD and WT select habitats that are conducive to their respective strategies: WT prefer flat terrain under tree cover that promotes high speed escape, while MD prefer open, mixed-terrain slopes that may be difficult for predators to navigate (Wood et al. 1989). In this way, the anti-predator strategies of each species dictate and compartmentalize their habitat use, alleviating the need to compete for resources in a shared space.

Mule X white-tailed deer hybrid zone in Alberta

In 1968, Charles Remington predicted the locations of 13 major “suture zones” in North America: areas that may be hotspots for hybridization activity due to locations of glacial refugia, anthropogenic disturbances, and various other biogeographic factors (Remington 1968). One suture zone of note that was later confirmed as a cluster of hybrid zones lies on the eastern foothills of the Rockies where the mountains meet the Great Plains (Swenson

and Howard 2005). Along this suture zone, from Alberta to Texas, mule and white-tailed deer exist in sympatry and anecdotal reports of hybridization are not unusual (Hornbeck and Mahoney 2000; Cronin 1988; Hughes and Carr 1993; Wishart 1980). These sightings, often hunter observations of deer with intermediate gross morphology, sparked the interest of researchers seeking to forecast the evolutionary outcomes of the local deer populations' hybridization tendencies.

Some morphological and physiological markers are helpful indicators of species membership. The most consistent seems to be the metatarsal gland, located on the outside of the lower half of the rear legs. MD metatarsal glands sit high on the lower leg and are about 10cm in length whereas the same gland in WTs sits lower, near or below the midpoint of the lower leg, and is less than 5cm in length (Stelfox 1993). F1 hybrid metatarsal glands are intermediate in both size and position (Carr et al. 1986; Wishart 1980). Another species-exclusive characteristic is the escape gait: the MD stot is highly differentiated from the WT gallop. Lingle (1993) conducted a detailed evaluation on the biomechanics of the preferred mode of escape for MD, WT, and their hybrids and found that hybrids do not closely replicate either the stot or the gallop. Instead, the hybrid "bound" showed elements of both and was largely inconsistent even within the same individual. Hybrid gaits were ultimately less efficient than either parental gait, leading the study to conclude that hybrids likely face a higher risk of predation. The metatarsal gland and escape gait biomechanics are both accessible, convenient, and reliable markers, but neither has proven to be consistent in post-F1 backcross hybrids.

Captive breeding studies have shown hybrid fertility to be drastically impaired, especially in males (Wishart et al. 1988). This is in accordance with Haldane's rule, which predicts infertility in hybrids of the heterogametic sex (Haldane 1922). In deer, like most mammals, males are hemizygous at X-linked loci. Without a second copy of the X chromosome to mask deleterious alleles, all X-linked Dobzhansky-Muller incompatibilities are expressed (Gompert et al. 2017). Despite apparent underdominance, female F1 hybrids can produce viable offspring (hybrids bred in captivity by Wishart et al. 1988 are analyzed in chapter 3). Fertility of only female hybrids allows for the possibility of backcrossed hybrids, the result of a mating between an F1 and a pure species. For example if a female F1 were to reproduce with a male MD, the offspring genomes would contain both MD alleles and WT alleles in a roughly 3:1 ratio. On average, the interspecific genetic background composition of a backcrossed hybrid genome will match the pedigree ancestry: three-quarters of the backcross MD genome comes from MD origin because three out of four grandparents are MD. As the lineage continues to backcross, the proportion of genomic ancestry of the introgressing species is halved at each generation; if the backcross MD should mate with a pure MD, the offspring genomes will be seven-eighths MD alleles and one-eighth WT alleles. This pattern may continue indefinitely, potentially redistributing heterospecific alleles across species, and creating a hybrid landscape with genomic gradients ranging from pure species to heavily introgressed lineages (Gompert and Buerkle 2016).

But without considerable resolution power, most hybrid activity will remain cryptic. Note that, in the above example, the WT ancestry decreases logistically with every generation. This makes hybrid detection a data-intensive process that requires substantial diagnostic

power to discern backcross generations (Oliviera et al. 2015). Consider also our focal system, where WT x MD F1 hybrids likely experience reduced fitness but females are able to reproduce. With each subsequent backcross generation, genomic ancestry resembles the pure species genome a little more. We therefore expect fitness to return incrementally because each backcross reduces the proportion of introgressed genome, suppressing negative epistatic interactions caused by incompatible allele combinations (Burke and Arnold 2001). If backcrossed hybrids are more fit than F1's, they will have greater ability to persist in wild populations, which may be reflected in relative abundances. Without the ability to recognize them, we forgo perhaps the most crucial aspect of the hybrid zone. Previous studies have largely relied on molecular markers targeting one or a few species-informative loci. A single diploid locus can indicate a pure species (homozygous) or an F1 (heterozygous) but, at minimum, post-F1 backcrosses require two loci to be identified. For example, Ballinger et al. (1992) employed both mtDNA restriction site mapping and a nuclear allozyme locus to identify several hybrids, all of which appeared to be backcrossed to some degree because all possessed a hybrid genotype at one locus but not the other. Studies with a broader selection of loci limit their reliance on any one locus and enjoy increased diagnostic power, which is imperative for the detection of deeper levels of introgression. I sought to improve hybrid diagnostic power by developing an assay of highly species-discriminating SNPs.

Chronic wasting disease considerations

MD and WT—as well as moose and elk—are affected by chronic wasting disease (CWD), a transmissible and fatal neurodegenerative disorder. Similar to scrapie in sheep

and bovine spongiform encephalopathy in cattle, CWD is caused by self-propagating, proteinaceous, infectious agents called prions (Colby and Prusiner 2011; Collinge 2001; Ma and Wang 2014). CWD is highly communicable and can spread directly or indirectly. The dominant mode of transmission is thought to be horizontal via direct physical contact and alimentary shedding of prions in excreta (Miller and Williams 2003), though vertical transmission is also common (Nalls et al. 2013). It can also persist in the environment without a living host for years, especially near infected carcasses (Miller et al. 2004). For this reason, caution should be used when managing land frequented by infected herds to prevent indirect exposure (Miller et al. 2000). The characterization of CWD transmission is an ongoing research effort that continues to add valuable insights for modelling disease dynamics and developing reliable diagnostic tests.

Tissues that are most sensitive to CWD diagnosis are only accessible postmortem, including the brainstem and medial retropharyngeal lymph node, but efforts are currently underway to develop antemortem diagnostic tests that are cost-effective and sensitive enough to use routinely on wild and captive populations (Haley and Richt 2017). Antemortem diagnosis is complicated by an incubation period of variable length; clinical symptoms typically take two to four years from the time of exposure to manifest and levels of infectious prions may be undetectable during the early pre-clinical phase (Williams et al. 2002; Williams 2005). Moreover, the disease may still be communicable during this time (Tamgüney et al. 2009). Cryptic transmission by pre-clinical animals can seriously hamstring containment efforts. CWD is currently endemic in two Canadian provinces, Alberta and Saskatchewan, and at least 23 states in the continental United States, including some near the Canadian

border such as Minnesota, Wisconsin, Michigan, Pennsylvania, New York, and both Dakotas (Haley and Hoover 2015; Richards 2018; see also https://www.cdc.gov/prions/cwd/occurrence.html). Containing the spread of CWD continues to be a high priority for the welfare of North American cervids.

My goals for this study were to (i) design a genomic resource capable of identifying advanced-generation backcrosses, (ii) implement that resource in a comprehensive survey of wild deer populations in Alberta, Canada, and (iii) investigate the pervasiveness of CWD among hybrid deer. A robust assessment of the current state of hybrids in wild populations will be useful to wildlife managers and governments seeking to optimize hunting policy and evaluate the potential evolutionary outcomes of hybridization. As well, CWD rates in hybridized deer will add to the ongoing research effort to characterize aspects of interspecific disease transmission; the incidence of CWD in hybrids may reveal the propensity for the disease to cross the species barrier. I've accomplished these objectives using a panel of 40 diagnostic single nucleotide polymorphisms to genotype individuals selected by a sampling method that accounted for CWD status, sex, and geographic location. I then genetically evaluated hybridity using *NewHybrids* version 1.1 (Anderson and Thompson 2002) and the R package *introgress* (Gompert and Buerkle 2010; Buerkle 2005).

Chapter 2: Development of a novel mule deer genomic assembly and species-diagnostic SNP panel for assessing introgression in mule deer, white-tailed deer, and their interspecific hybrids2

Introduction

Hybridization is not uncommon among plant and animal taxa worldwide (Schwenk *et al.* 2008). Mallet (2005) estimated that 10-30% of multicellular plant and animal species hybridize regularly and that, of those species, between 1 in 100 and 1 in 10 000 sympatric individuals are hybrids. Introgression has been identified as a mechanism for generating progressive evolutionary events such as novelty, divergent selection, and speciation (Dowling and Secor 1997; Lamer *et al.* 2015; Seehausen 2004) though it can also complicate management and conservation by compromising coadapted gene complexes (Edmands *et al.* 2009), morphological discernment (Leary *et al.* 1996), local adaptation (Martinsen *et al.* 2001), and the genetic integrity of unique phylogenetic lineages (Rhymer and Simberloff 1996). For these reasons, genetic tools to monitor current distributions and degrees of hybridization will be valuable for future researchers, policy-makers, hunters, and conservationists.

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2 Chapter 2 was published in the March 2019 edition of G3: Genes|Genomes|Genetics (doi: 10.1534/g3.118.200838). Since I led the authorship of the original manuscript, I've included the full article here as it appears in the journal. The mule deer genomic assembly was entirely the work of our collaborators, Arun Kommadath, Paul Stothard, and Allen Herbst. The species-diagnostic SNP panel discussed in this chapter was developed with assistance from their genomic assembly. The article is included here in its entirety for clarity and continuity purposes.

Introgressive hybridization tends to occur most frequently among sympatric, closely related species in rapidly diversifying adaptive radiations (Abbott *et al.* 2013; Gourbière and Mallet 2010; Price and Bouvier 2002) such as between mule deer (MD) (*Odocoileus hemionus*) and its sister species, white-tailed deer (WT) (*O. virginianus*), in the prairies of Western North America (Bradley *et al.* 2003 ; Cronin 1991; Hornbeck and Mahoney 2000; Stelfox and Adamczewski 1993). Cervids constitute cornerstone taxa in ecological, economic, and cultural sectors of Western Canada. The output value of elk and deer farms, before indirect spillover effects into other industries, is estimated to be more than $43 million in Canada with Alberta being responsible for over a quarter of that total (Petigara *et al.* 2011). As well, total big game hunting expenditures by Canadian residents in 2012 exceeded $1 billion with $169 million coming from Albertans (Federal, Provincial, and Territorial Governments of Canada, 2014). As of 2018, Canadian federal and provincial laws do not recognize hybrid deer as a separate entity from the parental species, which can create confusion as to hunting and harvesting regulations. Access to tools capable of identifying hybrid individuals and characterizing the rate of hybridization in wild populations could facilitate the implementation of management standards. Hybridization may also play a role in the spread of chronic wasting disease (CWD), a transmissible spongiform encephalopathy (TSE) that affects both focal *Odocoileus spp.* (Miller *et al.* 2012; Williams 2005). CWD has been reported to be more prevalent in MD relative to WT in areas of sympatry (Habib *et al.* 2011; Miller *et al.* 2000). Behavioral differences between species, in part, account for this asymmetry (Cullingham *et al.* 2010; Cullingham *et al.* 2011) but genetic polymorphisms have also been implicated (Wilson *et al.* 2009). Considering that hybrids potentially provide

an opportunity to bridge disease transmission across species, further research of CWD susceptibility and pervasiveness among hybrids may provide insight into the transmission dynamics.

Identification of hybrid deer using morphological traits alone is rarely done with confidence. Coloration and antler shape are not always intermediate between parental phenotypes. The most consistent and accessible morphological marker appears to be the metatarsal gland, which is intermediate in both position and size (Carr *et al.* 1986; Wishart 1980). The biomechanics of the escape gait may serve as another indicator. While the stot of the MD is highly differentiated from the gallop of the WT, the bound of the hybrid is highly variable, even between strides of the same individual, and seems to be wholly inefficient (Lingle 1993). Molecular markers used to identify hybrids include serum albumin electrophoresis (Hornbeck and Mahoney 2000), a ribosomal 28S DNA marker (Bradley *et al.* 2003), and mitochondrial endonuclease recognition site mapping (Carr *et al.* 1986). None of these methods, however, are reliably informative of backcrosses after the F1 generation. Single nucleotide polymorphisms (SNPs) have proven effective in investigations of admixture between divergent taxa in several hybrid systems (Stephens *et al.* 2009; Twyford and Ennos 2011; Wiley *et al.* 2009). This is due, in part, to their high abundance in the genome which improves discriminating power and facilitates the recognition of varying levels of introgression. Furthermore, SNPs are more consistently reproducible, easier to automate, and more stable in mammals than microsatellites (Fernandez *et al.* 2013; Tokarska *et al.* 2009). An added benefit of SNPs is that their biallelic nature is convenient for the discernment of two different species.

A species-discriminating SNP assay will effectively describe two traits of hybridization: the classification of hybrids based on the presence of species-specific alleles and a measure of hybridity based on the proportions of those alleles. Quantification of the varying introgression depths of hybridized populations will provide a snapshot with more resolution than would be available from observation of the F1 individuals alone. Detection power of this magnitude is necessary to explore hybrid zone structure because current hypotheses predict reduced fitness in F1 individuals (Hornbeck and Mahoney 2000); therefore, the frequency and abundance of F1's in the wild is not a suitable proxy for the occurrence of advanced-generation backcrosses. The ability to resolve backcross generation status will be informative of hybrid productivity and directionality. By differentiating advanced-generation backcrosses from pure breeding individuals, the rate of false negative hybrid diagnoses will drastically decrease, effectively increasing hybrid sample size and lineage diversity as well as our understanding of hybridization frequency and geographical distribution (Lamer *et al.* 2015). The identification of the degree of introgression is crucial for determining the structure and stability of the population and the entire hybrid zone in which it resides. For example, whether selection is working for or against hybrids can help to elucidate the type of suture zone present and, from that information, we can draw inferences and predictions about the hybrid zone itself (Hu 2005). By better understanding the structure of the populations that make up the hybrid zone, we improve our ability to forecast its future.

Currently, molecular resources suitable for application in *Odocoileus spp.* exist as sets of various individual loci but none capture mass sequence data capable of providing a reference to which polymorphisms can be mapped. Along with assorted microsatellite loci

(Bishop *et al.* 1994; DeWoody *et al.* 1995; Jones *et al.* 2000; Wilson *et al.* 1997), current SNP datasets have been engineered by subjecting MD, black-tailed deer (*O. h. columbianus*), and WT to genotyping on the BovineSNP50 Bead Chip (Haynes and Latch 2012); bovine exon-targeted resequencing (Powell *et al.* 2016); and mapping high-throughput next-generation sequencing data to an existing bovine reference genome assembly (Brauning *et al.* 2015). All of these datasets, however, relied heavily upon previously available bovine genomic resources for mapping and/or template purposes, thereby losing data in regions not conserved after the divergence of Cervidae and Bovidae some 28 million years ago (Hassanin *et al.* 2012). A whole genome sequence (WGS) assembly of MD will function as a more suitable reference for future polymorphism mining in Cervids and increase accessibility to regions of the genome not conserved from Bovidae.

In this study, we present two novel genomic resources targeted for the study of MD and MD X WT hybrids: a draft WGS assembly of the MD and a species-diagnostic SNP panel. The MD WGS data is a versatile and informative dataset for cervid researchers investigating the genetic basis of traits, disease susceptibilities, and variants by providing a reference genome to which polymorphisms can be mapped (Ng and Kirkness 2010). The species-diagnostic SNP panel provides a method to reliably differentiate between MD and WT as well as to identify hybrids and quantify introgressive geneflow, even in the limited amounts present in advanced-generation backcrosses.

Materials and methods

Reference-guided MD genome assembly and annotation

Total DNA was isolated from the ear tissue of a hunter harvested MD. The ear was shaved to remove hair and subsequently powdered by grinding under liquid nitrogen with a mortar and pestle. DNA was extracted from the powdered ear tissue (0.1g) for one hour at 68 degrees centigrade with 500µl of an extraction buffer containing 2% cetrimonium bromide, 100mM TrisCl pH7.5, 1.4M NaCl, 20mM EDTA, 2% polyvinylpyrrolidine, 0.2% mercaptoethanol, 100µg/mL proteinase K, and 20µg/mL RNase A. An equal volume of chloroform/isoamyl alcohol (24:1) was added, the sample centrifuged for 5 minutes at 14,000 x g and the aqueous phase collected. The aqueous phase was extracted with tris saturated phenol/chloroform, centrifuged and again the aqueous phase collected. Finally, the DNA was precipitated with 2.5 volumes of 95% ethanol and 0.1 volume of sodium acetate. The pellet was washed with 70% ethanol, briefly dried to remove residual ethanol, and dissolved in water. DNA quality was assessed by UV-Vis spectroscopy and agarose gel electrophoresis.

Genomic DNA was sequenced on an Illumina HiSeq X Ten sequencer (shotgun PCR free library; paired-end sequencing; 150 bp reads). Read quality was assessed using FastQC software (available at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) prior to and after performing quality control (QC) steps. The QC included quality based read trimming and adapter removal using Trimmomatic (Bolger *et al.* 2014) followed by adapter and phiX read removal using Cutadapt (Martin 2011) with default settings for both software. Reads that passed QC were mapped to the WT deer reference genome assembly (Ovir.te_1.0; GenBank assembly accession: GCA_002102435.1) using the BWA-MEM algorithm of the Burrows-Wheeler Alignment tool (Li 2013) with default settings. Mapped

reads were sorted and indexed using SAMtools (Li *et al.* 2009). Duplicate reads were tagged using MarkDuplicates software from Picard tools (available at: http://broadinstitute.github.io/picard). Variant calling and MD consensus sequence generation were performed using a combination of mpileup, bcftools and vcfutils.pl scripts from SAMtools. Identified SNPs were annotated using SnpEff (Cingolani *et al.* 2012). Regions with no coverage on the consensus sequence were filled with N's.

Selection and validation of species-discriminating SNPs

DNA was extracted from a sampling group of multiple species of deer as part of another study whose purpose was to aggregate and identify markers that would be useful to the New Zealand deer industry for parentage assignment and sub-species differentiation (Rowe *et al.* 2015). Samples were genotyped on Illumina 50K CervusSNP50, 24-sample Bead Chips (Illumina, San Diego), a high-throughput SNP assay developed for deer (*Cervus* genus) by comparing genomic sequence data from red deer, wapiti (subspecies of *Cervus elaphus*), and sika (*Cervus nippon*) by Brauning *et al.* (2015). A set of 44,448 SNPs were genotyped in 396 individuals, including 17 WT and 8 MD from Alberta and Saskatchewan, Canada. Loci and samples exhibiting non-autosomal and non-Mendelian inheritance patterns, low call rates, and duplication were excluded (Rowe *et al.* 2015). The GenABEL package in R software (Aulchenko *et al.* 2007) was used to carry out genotype quality control measures. We identified 129 loci that had call rates >0.7 in both WT and MD and also appeared to show fixed differences between the two species, ie. biallelic loci where one allele is homozygous in WT and the other in MD.

A preliminary validation step was implemented by aligning the 201 bp sequences that include the SNP and its 100 bp flanking sequences to the new MD genome assembly generated here and to the WT genome assembly (Ovir.te_1.0). This allowed us to eliminate 10 SNPs that were non-discriminating or otherwise unable to be mapped prior to assay design and further validation, leaving 119 species-discriminating loci, from which a subset of 40 were randomly selected for validation. Because the morphology of hybrids varies and their frequency in wild populations as of now can only be approximated, the genetic purity of deer from Alberta should not be assumed. Even minute levels of introgression in individuals believed to be pure could compromise the efficacy of species delimitation. As a precaution, we minimized the risk of ancestral hybridization by importing samples from regions of allopatry, where hybridization is less common or non-existent. MD are native to western North America; populations are rarely found further east than western Minnesota or Iowa (Patterson *et al.* 2003). For this reason, we used WT samples collected in Ontario (n=10). WT distribution spans continental North America but excludes most of Utah and Nevada (Hewitt 2011). MD samples used for validation were collected from Utah (n=2), Nevada (n=5), and Montana (n=3). Note that Montana is a known region of sympatry and thus should be considered as quantitative support of the other two MD sampling locations but would otherwise not be used independently. As well, samples of *a priori* hybrid heredity (n=10) were obtained from Alberta Environment and Parks. Pedigree records were available for these individuals, as they were intentionally bred as hybrids as part of a long-term study in the mid-1980's (Wishart *et al.* 1988). DNA was extracted using a Qiagen 96 DNeasy® Blood

and Tissue kit following the manufacturer's instructions (Qiagen, Mississauga, Ontario, Canada) and genotyped on the final panel.

SNP assay assignment efficacy

HybridLab software (Nielsen *et al.* 2006) uses classical genetics principles to simulate offspring genotypes from a set of observed parental genotypes. Two populations of pure and hybrid genotypes were independently simulated in *HybridLab* using the parental genotypes of 10 pure MD from Utah, Nevada, and Montana and 10 pure WT from Ontario. Simulated populations consisted of 100 individuals in each of 10 hybrid generation classes: parental WT, parental MD, F1, F2, and three backcross generations of each species. Posterior probabilities of assignment for simulated populations were computed by *NewHybrids* version 1.1 (Anderson and Thompson 2002) using 500,000 burnin iterations preceding 500,000 sweeps. Genotype frequency classes were set in *NewHybrids* using Jeffrey's-like priors (table 1). Repetitions were also done using two and four backcross generations (data not shown): fourth backcrosses could not be distinguished from third backcrosses or pure-species and, upon retraction of the third backcross as an assignment option, the post-secondary backcross individuals were erroneously assigned to the second backcross generation. For these reasons, the third backcross generation was deemed as the most advanced introgression level detectable[3]. *NewHybrids* assignments were tested for efficacy using the R package *hybriddetective* (Wringe *et al.* 2017). *hybriddetective* is designed to analyze hybrid assignments as deep as one backcross generation, therefore, some code

3 See chapter 3 for improved evaluation of fourth generation backcrosses using more relaxed introgression estimating methods.

was amended to account for two additional levels of introgression (available upon request). Assignment probabilities for simulated populations, as calculated by *NewHybrids,* were evaluated by *hybriddetective* using three metrics: accuracy, the proportion of assignments to a particular category that were correct ([correct assignments] / [total assignments of a particular category]); efficiency, the proportion of individuals in a particular category that were assigned correctly ([correct assignments] / [total individuals of a particular category]); and power, the product of accuracy and efficiency (Vähä and Primmer 2006).

Results

MD genome assembly, variant identification and annotation

A total of 378,654,417 paired-end reads were obtained in fastq format files. Following quality control steps, 334,409,387 paired-end reads remained that were then aligned to the WT reference genome assembly. A total of 536,357,624 reads were mapped (mapping rate of 75%) of which 81.69% were properly paired. The average genome-wide coverage and GC content of mapped reads were 25X and 40% respectively. Variant calling on mapped reads compared to the WT reference resulted in approximately 30 million SNPs and 2.9 million INDELs. After filtering at SNP mapping quality Q20, the variants ranged from 30.1M at read depth 10 to 32.9M at read depth 2. The genome-wide ratio of transitions to transversions at read depth 5 and Q20 was 2.4, which is similar to the value reported in human studies (DePristo *et al.* 2011; Jun *et al.* 2015).

Species-discriminating SNP assay

Based on aligning the selected loci and their 100 bp flanking sequences to the genome assembly of the Red Deer (CerEla1.0, GenBank: GCA_002197005.1), which is finished at

chromosomal level, we determined that the 40 loci were distributed across 24 of the 35 Red deer chromosomes (fig.1), indicating good genome-wide distribution. SNP genotypes of 30 individuals, including pure MD, pure WT, and *a priori* hybrids, were successfully called at all 40 loci. Because all loci were highly discriminating, pure-breeding, F1, and F2 individuals were assigned with predictable confidence: all reached 100% efficiency and greater than 93% accuracy at a critical posterior probability threshold of 0.50. Likewise, first-generation backcrosses were detected at ~95% efficiency and accuracy at the same threshold. Second- and third-generation WT backcrosses were assigned with slightly more proficiency than their MD counterparts; advanced-generation WT backcross assignments were accurate and efficient at a rate of ~85%, while the same MD assignments achieved rates of ~75%. Accuracy increased with the probability threshold, but efficiency and sample size decreased (fig.2). Both of these trends agree with expected outcomes. The critical probability threshold will be subjective based on the user's research question; by increasing the threshold, some samples assigned with a lower probability will be excluded but those that remain are more likely to be assigned correctly. For all samples to be assigned to a hybrid category, a probability threshold of 0.5 should be used. The power of assignment for a specific hybrid category slowly declined as the probability threshold was increased from 0.5 to 0.8, then decreased more drastically. Alternatively, the power of assignment for hybrids in general (i.e. all hybrid categories combined) remained relatively stable over a wider range of probability thresholds: 0.80 at a critical probability threshold of 0.5 and 0.69 at a threshold of 0.9.

Discussion

The development of the MD genome assembly helped us to identify fixed differences between WT and MD, and to develop a 40-loci SNP assay capable of reliably classifying pure WT, pure MD, and their interspecific hybrids into introgression categories. This diagnostic panel overcomes several limitations encountered by previous methods used in deer hybridization analyses, including: lack of assignment power due to low marker abundance, ambiguity of distinguishing morphological traits, inconsistent reproducibility, and inability to resolve advanced-generation backcrosses. Along with a solution to these deficiencies, the whole genome sequence, to which these and other polymorphisms can be mapped, improves accessibility to regions of the MD genome previously withheld from analyses by divergence from model research species. Together, these resources bear valuable conservation potential for researchers, hunters, farmers, and environmentalists.

Hybrid populations simulated in duplicate using genotypes of pure-species from regions of allopatry demonstrated that the SNP assay diagnostic strength is reliable up to and including the third backcross generation. Simulations were composed of 10 hybrid classes (n=100 for each class), including both pure species, F1 and F2 hybrids, and 3 backcross generations of each species (Table 1). We chose not to include simulations for individuals with pedigrees containing multiple hybrids (e.g. backcross X backcross or backcross X F1/F2 scenarios) because the differences in individual genotype frequencies are very low between respective classes and any attempt to distinguish those classes with medium-throughput genotype data would be impractical. Furthermore, Wishart *et al.* (1988) found male F1 hybrids to be almost exclusively sterile, significantly decreasing the likelihood of

encountering, in a wild population, an F2 or other product of hybrid X hybrid pairings. This apparent adherence to Haldane's rule (Haldane 1922), which predicts infertility in the heterogametic sex of interspecific hybrids, is just one opportunity for future research in a complex hybrid system that now has the molecular resources for further investigation. Another question of interest is the directionality of hybridization. In the same study, more viable offspring were produced from crosses under controlled laboratory conditions between MD males and WT females than in the reciprocal cross (Wishart *et al.* 1988); however, studies of hybrids in wild populations have both supported this finding (Carr *et al.* 1986) and refuted it (Carr and Hughes 1993; Cathey *et al.* 1998; Stelfox and Adamczewski 1993). Behavioral traits such as boldness and promiscuity likely play major roles in this dynamic (Lingle *et al.* 2007a; Lingle, *et al.* 2007b) but genetic incompatibilities may also contribute, as indicated by increased spontaneous abortions of fetuses generated by MD female X WT male matings (Wishart *et al.* 1988). This assay will be instrumental in validating observations such as these with empirical data from the field by alleviating reliance on pedigree records. By confirming or disproving the persistence of these trends in wild populations, we can explore the structural dynamics of the hybrid zone with more resolution than has been available in the past. Furthermore, the focal hybrid system is not confined to western Canada; studies have reported MD x WT hybridization in West Texas and Montana (Carr *et al.* 1986; Cronin 1991). Whether these zones are consistent or clinal in hybridization frequency is yet to be investigated. Rusek *et al.* (2015) noted that, although hybrid complexes are not uncommon in nature, their evolutionary inception and subsequent persistence remains relatively ambiguous and that studies targeted towards fine-scale

genetic analyses of such systems are largely limited by lack of resources capable of identifying and quantifying introgression.

Previous sets of molecular markers and a few diagnostic morphological traits were successful at identifying F1 hybrids with some degree of accuracy but none had the diagnostic power to differentiate subsequent backcross generations; many backcross hybrids were mistaken either as F1's or as pure-species. Without the ability to interrogate more advanced generations, the underlying factors balancing the co-existence of hybrids with parental species has remained inaccessible to researchers and conservationists (Hornbeck and Mahoney 2000). Such factors are both ecological and genetic in nature and typically include reproductive barrier strength, vigour and fertility of hybrids, pathogen pressure, and selection by predation (Daum *et al.* 2012; Duenez-Guzman *et al.* 2009; Hall *et al.* 2006; Wolf *et al.* 2001). The first step to elucidating the specific conditions of these factors as they pertain to the focal system is to delve deeper into the structure and distribution of hybridized populations via a large-scale study. With access to advanced-generation backcrosses, we now have the detection power necessary to capture a robust snapshot of the hybrid landscape. Population data will be imperative to resolve broad questions about the hybrid zone in which the *Odocoileus* system resides. For example, which model does it most resemble? Endogenous selection against hybrids (heterozygote breakdown) is indicative of a tension zone, whereas exogenous selective pressure potentially driven by niche divergence in the parental species is more consistent with the geographical selection-gradient model (Johnson *et al.* 2016). A third possibility would be that hybrids fill some ecological niche and are being selected for. Until a population-scale study is undertaken, we

can only theorize. The results of that study will not only advance the collective body of knowledge of hybrid zones, but more generally, that of cervid evolution and ecology in western North America at a time when they are faced with the looming threat of chronic wasting disease. CWD is a fatal neurodegenerative disorder that has proven highly transmissible in wild populations (Williams *et al.* 2002). At the time of this writing, CWD is endemic in two Canadian provinces, Alberta and Saskatchewan, and at least 23 states in the continental United States, including some near the Canadian border such as Minnesota, Wisconsin, Michigan, Pennsylvania, New York, and both Dakotas (Haley and Hoover 2015; Richards 2018; see also https://www.cdc.gov/prions/cwd/occurrence.html). Containing the spread of CWD continues to be a high priority for governmental bodies, researchers, and conservationists because of the cultural, economic, and ecological importance of cervids in Canada and the United States.

The MD whole genome sequence presented here may also serve to progress the study of CWD. Genetic polymorphisms of the *Prnp* gene, which encodes the protein that is eventually converted into the infectious agent, have been associated with varied disease incubation periods (Wilson *et al.* 2009). Our assembly provides an ideal reference to which these and other variable sites can be mapped, while also serving as a localizing template for structures of interest, such as primer design. The utility of this functional, all-purpose *Odocoileus* reference lies in alleviating the reliance on model-species genomes that may be less recently diverged. In doing so, ease of access to novel regions of the genome, including those not conserved in Bovidae and undocumented subtleties such as promoters, splice sites, and introns, will be vastly improved. Facilitating access to these untapped domains will

effectively foster the development of more specific molecular advances applicable to a range of research questions throughout the *Odocoileus* genera.

Table 1. Assignment criteria and category definition of white-tailed (WT) and mule deer (MD) hybrids. Heterozygous and homozygous genotype frequencies are required as input parameters to *NewHybrids* to specify the number and configuration of hybrid classes.

		Genotype frequency			
NewHybrid category		WT (AA)	H (AB)	MD (BB)	NewHybrid category expanded
WT	Parental WT	1	0	0	WT
MD	Parental MD	0	0	1	MD
F1	First-generation hybrid	0	1	0	(WT x MD)
F2	Second-generation hybrid	0.25	0.5	0.25	((WT x MD) x (WT x MD))
BxWT	First-generation backcross	0.5	0.5	0	(WT x (WT x MD))
BxMD	First-generation backcross	0	0.5	0.5	(MD x MD x WT))
Bx2WT	Second-generation backcross	0.75	0.25	0	(WT x (WT x (WT x MD)))
Bx2MD	Second-generation backcross	0	0.25	0.75	(MD x (MD x (MD x WT)))
Bx3WT	Third-generation backcross	0.875	0.125	0	(WT x (WT x (WT x (WT x MD))))
Bx3MD	Third-generation backcross	0	0.125	0.875	(MD x (MD x (MD x (MD x WT))))

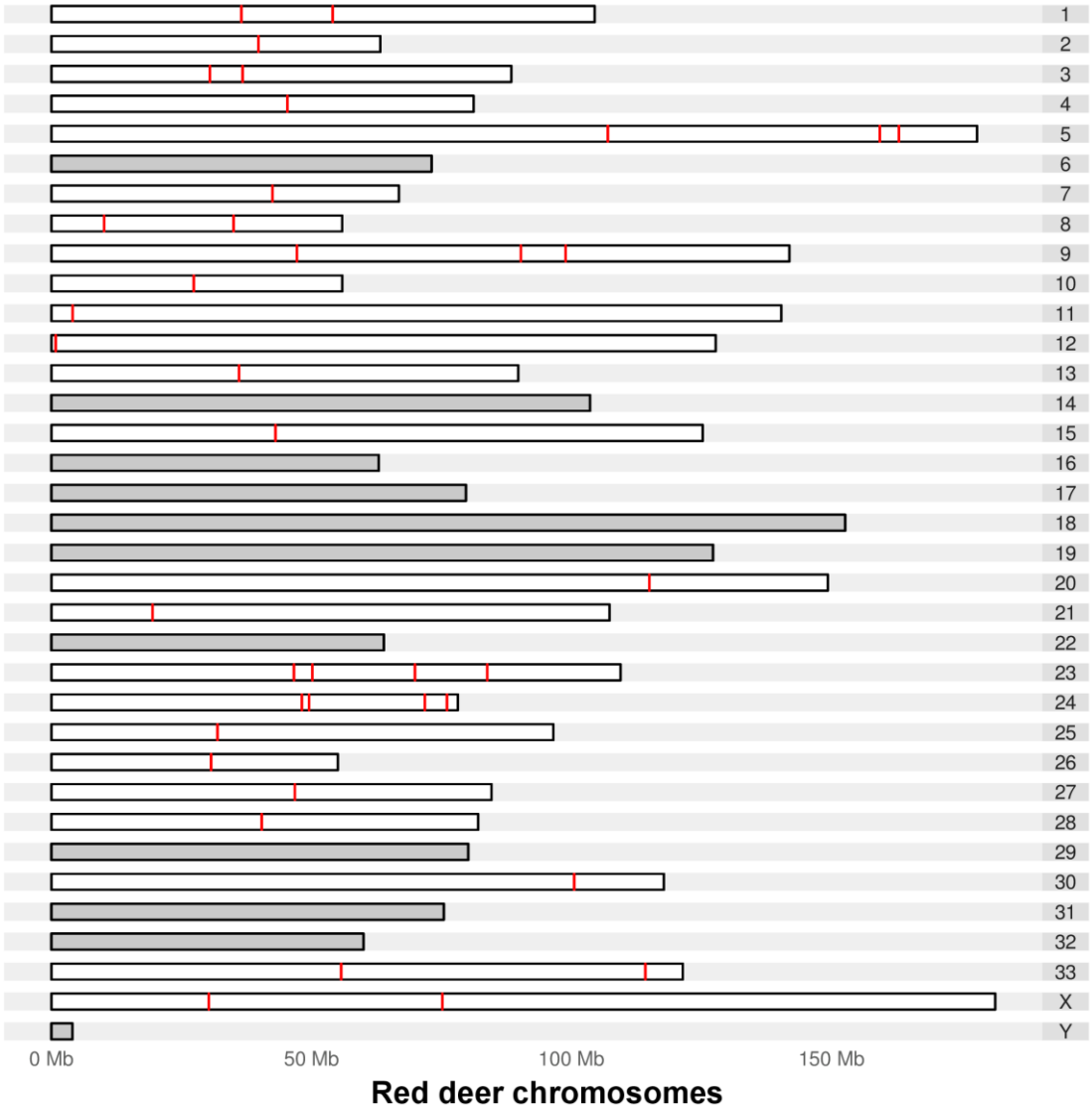

Figure 1. MD/WT species discriminating SNP loci mapped to Red Deer genome assembly. Red bars indicate the positions on the Red deer chromosomes that the 40 loci SNP assay mapped to. The mapping positions were determined by alignments of the SNP loci along with the 100 bp flanking sequences onto the Red deer genome assembly (CerEla1.0) using BLAT. The loci covered 24 of the 35 chromosomes, with some chromosomes harbouring multiple loci (chromosomes not covered are shaded grey). One of the loci mapped to an unplaced contig (not depicted here).

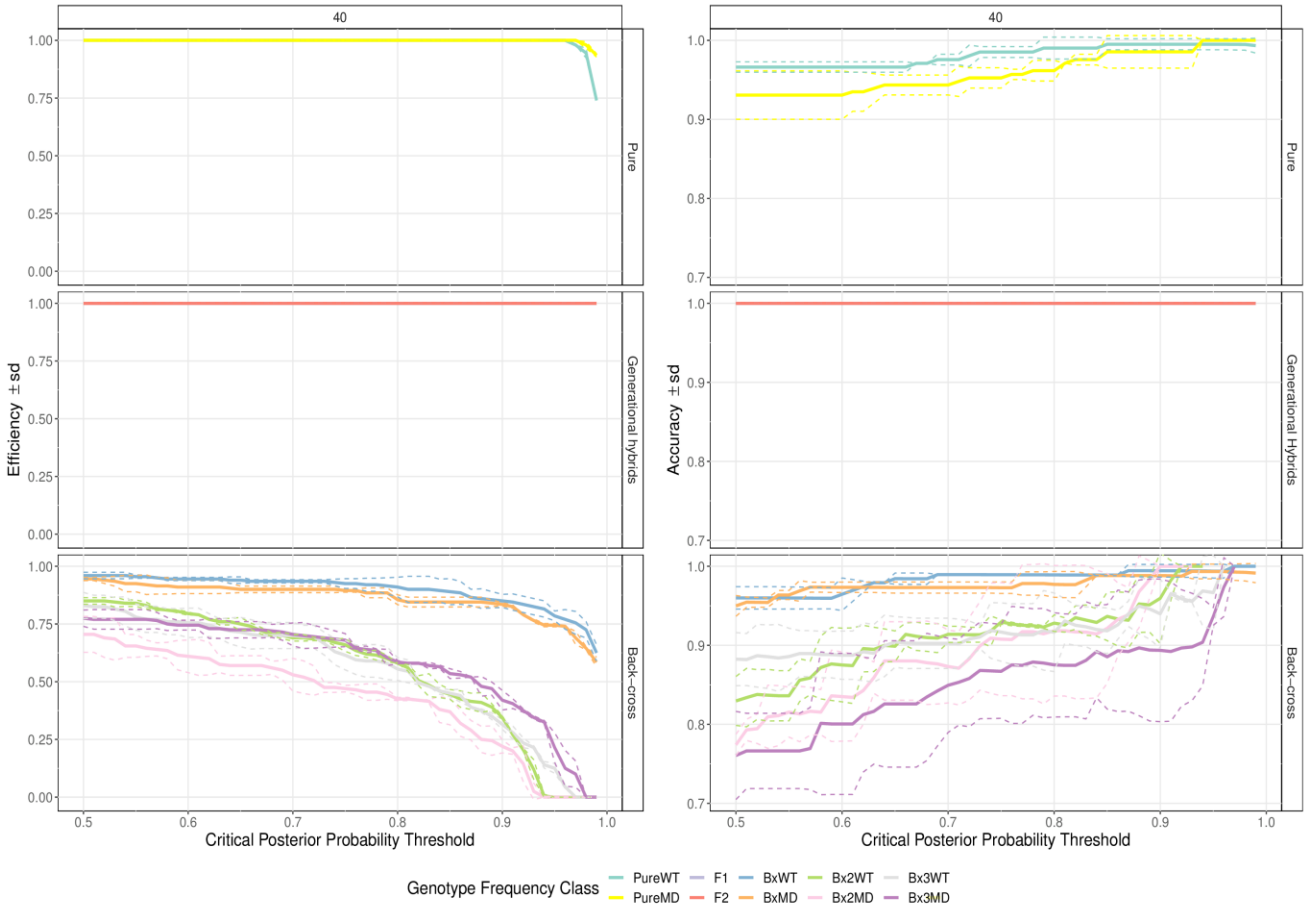

Figure 2. Hybrid assignment efficacy as determined by the hybridPowerComp function in the R package hybriddetective. Facets labelled as Pure indicate parental WT and MD, Generational Hybrids refers to F1 and F2 individuals (lines overlaid), and Back-cross includes first, second, and third generation backcrosses of both species. Accuracy was calculated as [correct assignments] / [total assignments of a particular category]. Efficiency was calculated as [correct assignments] / [total individuals of a particular category].

References

Abbott, R., Albach, D., Ansell, S., Arntzen, J. W., Baird, S. J. E., Bierne, N., … Zinner, D. (2013). Hybridization and speciation. *Journal of Evolutionary Biology, 26*(2), 229-246. doi: 10.1111/j.1420-9101.2012.02599.x

Anderson, E. C., and Thompson, E. A. (2002). A model-based method for identifying species hybrids using multilocus genetic data. *Genetics, 160*(3), 1217. http://www.genetics.org/content/160/3/1217.abstract

Aulchenko, Y. S., Ripke, S., Isaacs, A., and van Duijn, C. M. (2007). GenABEL: An R library for genome-wide association analysis. *Bioinformatics, 23*(10), 1294-1296. http://dx.doi.org/10.1093/bioinformatics/btm108

Bishop, M., Kappes, S., Keele, J., Stone, R., Sunden, S., Hawkins, G., … Beattie, C. W. (1994). A genetic-linkage map for cattle. *Genetics, 136*(2), 619-639. PubMed ID: 7908653

Bolger, A. M., Lohse, M., and Usadel, B. (2014). Trimmomatic: A flexible trimmer for illumina sequence data. *Bioinformatics, 30*(15), 2114-2120. doi: 10.1093/bioinformatics/btu170

Bradley, R. D., Bryant, F. C., Bradley, L. C., Haynie, M. L., Baker, R. J., and Jones, C. A. (2003). Implications of hybridization between white-tailed deer and mule deer. *The Southwestern Naturalist, 48*(4), 654-660. doi: 10.1894/0038-4909(2003)048<0654:IOHBWD>2.0.CO;2

Brauning, R., Fisher, P., Mcculloch, A., J Smithies, ,Russell, Ward, J., Bixley, M., … McEwan, J. (2015). Utilization of high throughput genome sequencing technology for large

scale single nucleotide polymorphism discovery in red deer and canadian elk. *bioRxiv 027318*. https://doi.org/10.1101/027318.

Carr, S. M., Ballinger, S. W., Derr, J. N., Blankenship, L. H., and Bickham, J. W. (1986). Mitochondrial DNA analysis of hybridization between sympatric white-tailed deer and mule deer in west texas. *Proc Natl Acad Sci USA, 83*(24), 9576. doi: 10.1073/pnas.83.24.9576

Carr, S., and Hughes, G. (1993). Direction of introgressive hybridization between species of north-American deer (*Odocoileus*) as inferred from mitochondrial-cytochrome-B sequences. *Journal of Mammalogy, 74*(2), 331-342. doi: 10.2307/1382388

Cathey, J., Bickham, J., and Patton, J. (1998). Introgressive hybridization and nonconcordant evolutionary history of maternal and paternal lineages in north American deer. *Evolution, 52*(4), 1224-1229. doi: 10.2307/2411253

Cingolani, P., Platts, A., Wang, L. L., Coon, M., Nguyen, T., Wang, L., … Ruden, D.M. (2012). A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of drosophila melanogaster strain w(1118); iso-2; iso-3. *Fly*, *6*(2), 80-92. doi:10.4161/fly.19695

Cronin, M. A. (1991). Mitochondrial and nuclear genetic relationships of deer (*Odocoileus spp*.) in western north America. *Canadian Journal of Zoology, 69*(5), 1270-1279. doi: 10.1139/z91-179

Cullingham, C. I., Cooke, J. E., K., Dang, S., and Coltman, D. W. (2013). A species-diagnostic SNP panel for discriminating lodgepole pine, jack pine, and their

interspecific hybrids. *Tree Genetics and Genomes, 9*(4), 1119-1127. doi: 10.1007/s11295-013-0608-x

Cullingham, C. I., Merrill, E. H., Pybus, M. J., Bollinger, T. K., Wilson, G. A., and Coltman, D. W. (2010). Broad and fine-scale genetic analysis of white-tailed deer populations: Estimating the relative risk of chronic wasting disease spread. *Evolutionary Applications, 4*(1), 116-131. doi: 10.1111/j.1752-4571.2010.00142.x

Cullingham, C. I., Nakada, S. M., Merrill, E. H., Bollinger, T. K., Pybus, M. J., and Coltman, D. W. (2011). Multiscale population genetic analysis of mule deer (*Odocoileus hemionus hemionus*) in western Canada sheds new light on the spread of chronic wasting disease. *Canadian Journal of Zoology, 89*(2), 134-147. doi: 10.1139/Z10-104

Daum, J. M., Davis, L. R., Bigler, L., and Woodhams, D. C. (2012). Hybrid advantage in skin peptide immune defenses of water frogs (*Pelophylax esculentus*) at risk from emerging pathogens. *Infection Genetics and Evolution, 12*(8), 1854-1864. doi: 10.1016/j.meegid.2012.07.024

DePristo, M. A., Banks, E., Poplin, R. E., Garimella, K. V., Maguire, J. R., Hartl, C., ... Daly, M. J. (2011). A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nature Genetics, 43*(5), 491-498. doi:10.1038/ng.806

DeWoody, J., Honeycutt, R., and Skow, L. (1995). Microsatellite markers in white-tailed deer. *Journal of Heredity, 86*(4), 317-319. doi: 10.1093/oxfordjournals.jhered.a111593

Dowling, T., and Secor, C. (1997). The role of hybridization and introgression in the diversification of animals. *Annual Review of Ecology and Systematics, 28*, 593-619. doi: 10.1146/annurev.ecolsys.28.1.593

Duenez-Guzman, E. A., Mavarez, J., Vose, M. D., and Gavrilets, S. (2009). Case studies and mathematical models of ecological speciation. 4. Hybrid speciation in butterflies in a jungle. *Evolution, 63*(10), 2611-2626. doi: 10.1111/j.1558-5646.2009.00756.x

Edmands, S., Northrup, S. L., and Hwang, A. S. (2009). Maladapted gene complexes within populations of the intertidal copepod *Tigriopus californicus*? *Evolution, 63*(8), 2184-2192. doi: 10.1111/j.1558-5646.2009.00689.x

Federal, Provincial, and Territorial Governments of Canada. 2014. *2012 Canadian Nature Survey: Awareness, participation, and expenditures in nature-based recreation, conservation, and subsistence activities.* Ottawa, ON: Canadian Councils of Resource Ministers.

Fernandez, M. E., Goszczynski, D. E., Liron, J. P., Villegas-Castagnasso, E. E., Carino, M. H., Ripoli, M. V., … Giovambattista, G. (2013). Comparison of the effectiveness of microsatellites and SNP panels for genetic identification, traceability and assessment of parentage in an inbred angus herd. *Genetics and Molecular Biology, 36*(2), 185-U194. doi: 10.1590/S1415-47572013000200008

Gourbière, S., and Mallet, J. (2010). Are species real? The shape of the species boundary with exponential failure, reinforcement, and the missing snowball. *Evolution, 64*(1), 1-24. doi: 10.1111/j.1558-5646.2009.00844.x

Habib, T. J., Merrill, E. H., Pybus, M. J., and Coltman, D. W. (2011). Modelling landscape effects on density-contact rate relationships of deer in eastern alberta: Implications for chronic wasting disease. *Ecological Modelling, 222*(15), 2722-2732. doi: 10.1016/j.ecolmodel.2011.05.007

Haldane, J. (1922). Sex ratio and unisexual sterility in hybrid animals. *Journal of Genetics, 12*(2), 101-109. doi: 10.1007/BF02983075

Haley, N. J., and Hoover, E. A. (2015). Chronic wasting disease of cervids: Current knowledge and future perspectives. *Annual Review of Animal Biosciences, 3*(3), 305-325. doi: 10.1146/annurev-animal-022114-111001

Hall, R. J., Hastings, A., and Ayres, D. R. (2006). Explaining the explosion: Modelling hybrid invasions. *Proceedings of the Royal Society B-Biological Sciences, 273*(1592), 1385-1389. doi: 10.1098/rspb.2006.3473

Hassanin, A., Delsuc, F., Ropiquet, A., Hammer, C., van Vuuren, B. J., Matthee, C., Ruiz-Garcia, M., … Couloux, A. (2012). Pattern and timing of diversification of *Cetartiodactyla (Mammalia, Laurasiatheria*), as revealed by a comprehensive analysis of mitochondrial genomes. *Comptes Rendus Biologies, 335*(1), 32-50. doi: 10.1016/j.crvi.2011.11.002

Haynes, G. D., and Latch, E. K. (2012). Identification of novel single nucleotide polymorphisms (SNPs) in deer (*Odocoileus spp*.) using the BovineSNP50 BeadChip. *Plos One, 7(*5) doi: 10.1371/journal.pone.0036536

Hewitt, D. (Ed.). (2011). Biology and management of white-tailed deer (1st ed.). Boca Raton, FL: CRC Press.

Hewitt, G. M. (1988). Hybrid zones- Natural laboratories for evolutionary studies. *Trends in Ecology and Evolution, 3*(7), 158-167. doi: https://doi.org/10.1016/0169-5347(88)90033-X

Hornbeck, G. E., and Mahoney, J. M. (2000). Introgressive hybridization of mule deer and white-tailed deer in southwestern alberta. *Wildlife Society Bulletin, 28*(4), 1012-1015. doi: 10.2307/3783860

Hu, X. (2005). Tension versus ecological zones in a two-locus system. *Theoretical Population Biology, 68*(2), 119-131. doi: https://doi.org/10.1016/j.tpb.2005.02.003

Jeffery, N. W., DiBacco, C., Wringe, B. F., Stanley, R. R. E., Hamilton, L. C., Ravindran, P. N., Bradbury, I. R. (2017). Genomic evidence of hybridization between two independent invasions of european green crab (*Carcinus maenas*) in the northwest atlantic. *Heredity, 119*(3), 154-165. doi: 10.1038/hdy.2017.22

Johnson, S. E., Delmore, K. E., Brown, K. A., Wyman, T. M., and Louis, E. E., Jr. (2016). Niche divergence in a brown lemur (*Eulemur spp.*) hybrid zone: Using ecological niche models to test models of stability. *International Journal of Primatology, 37*(1), 69-88. doi: 10.1007/s10764-015-9872-y

Jones, K., Levine, K., and Banks, J. (2000). DNA-based genetic markers in black-tailed and mule deer for forensic applications. *California Fish and Game, 86*(2), 115-126. https://www.cabdirect.org/cabdirect/abstract/20013155304

Jun, G., Wing, M. K., Abecasis, G. R., and Kang, H. M. (2015). An efficient and scalable analysis framework for variant extraction and refinement from population-scale DNA sequence data. *Genome Research, 25*(6), 918-925. doi:10.1101/gr.176552.114

Lamer, J. T., Ruebush, B. C., Arbieva, Z. H., McClelland, M. A., Epifanio, J. M., and Sass, G. G. (2015). Diagnostic SNPs reveal widespread introgressive hybridization between introduced bighead and silver carp in the mississippi river basin. *Molecular Ecology, 24*(15), 3931-3943. doi: 10.1111/mec.13285

Lamer, J. T., Sass, G. G., Boone, J. Q., Arbieva, Z. H., Green, S. J., and Epifanio, J. M. (2014). Restriction site-associated DNA sequencing generates high-quality single nucleotide polymorphisms for assessing hybridization between bighead and silver carp in the united states and china. *Molecular Ecology Resources, 14*(1), 79-86. doi: 10.1111/1755-0998.12152

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

Leary, R. E., Gould, W. R., and Sage, G. K. (2011). Success of basibranchial teeth in indicating pure populations of rainbow trout and failure to indicate pure populations of westslope cutthroat trout. *North American Journal of Fisheries Management, 16*(1), 210-213. doi: 10.1577/1548-8675(1996)016<0210:SOBTII>2.3.CO;2

Li H. (2013) Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. Available from: http://arxiv.org/abs/1303.3997

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Martin, G. … Durbin, R. (2009). The sequence Alignment/Map format and SAMtools. *Bioinformatics, 25*(16), 2078-2079. doi: 10.1093/bioinformatics/btp352

Lingle, S. (1993). Escape gaits of white-tailed deer, mule deer, and their hybrids: Body configuration, biomechanics, and function. *Canadian Journal of Zoology, 71*(4), 708-724. doi: 10.1139/z93-095

Lingle, S., Rendall, D., and Pellis, S. M. (2007). Altruism and recognition in the antipredator defence of deer: 1. Species and individual variation in fawn distress calls. *Animal Behavior. 73*(5), 897-905. doi: 10.1016/j.anbehav.2006.11.003

Lingle, S., Rendall, D., Wilson, W. F., DeYoung, R. W., and Pellis, S. M. (2007). Altruism and recognition in the antipredator defence of deer: 2. Why mule deer help nonoffspring fawns. *Animal Behavior. 73*(5), 907-916. doi: 10.1016/j.anbehav.2006.11.004

Lohr, J. N., and Haag, C. R. (2015). Genetic load, inbreeding depression, and hybrid vigor covary with population size: An empirical evaluation of theoretical predictions. *Evolution, 69*(12), 3109-3122. doi: 10.1111/evo.12802

Mallet, J. (2005). Hybridization as an invasion of the genome. *Trends in Ecology and Evolution, 20*(5), 229-237. doi: 10.1016/j.tree.2005.02.010

Martin, M. (2011). CUTADAPT removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal, 17*(10-2) doi: 10.14806/ej.17.1.200

Martinsen, G., Whitham, T., Turek, R., and Keim, P. (2001). Hybrid populations selectively filter gene introgression between species. *Evolution, 55*(7), 1325-1335. doi: 10.1111/j.0014-3820.2001.tb00655.x

Miller, M. W., Wolfe, L. L., Sirochman, T. M., Sirochman, M. A., Jewell, J. E., and Williams, E. S. (2012). Survival patterns in white-tailed and mule deer after oral inoculation with a

standardized, conspecific prion dose. *Journal of Wildlife Diseases, 48*(2), 526-529. doi: 10.7589/0090-3558-48.2.526

Miller, M., Williams, E., McCarty, C., Spraker, T., Kreeger, T., Larsen, C., Thorne, E. (2000). Epizootiology of chronic wasting disease in free-ranging cervids in Colorado and Wyoming. *Journal of Wildlife Diseases, 36*(4), 676-690. doi: 10.7589/0090-3558-36.4.676

Ng, P. C., and Kirkness, E. F. (2010). Whole genome sequencing. *Genetic variation: Methods and protocols, 628*, 215-226. doi: 10.1007/978-1-60327-367-1_12

Nielsen, E. E., Bach, L. A., and Kotlicki, P. (2006). HYBRIDLAB (version 1.0): A program for generating simulated hybrids from population samples. *Molecular Ecology Notes, 6*(4), 971-973. doi: 10.1111/j.1471-8286.2006.01433.x

Patterson, B. D., Ceballos, G., Sechrest, W., Tognelli, M. F., Brooks, T., Luna, L., Ortega, P., Salazar, I., Young, B. E. (2003) Digital distribution maps of the mammals of the western hemisphere, version 1.0. NatureServe, Arlington, Virginia, USA.

Petigara, M., Dridi, C., and Unterschultz, J. (2011). The economic impacts of chronic wasting disease and bovine spongiform encephalopathy in Alberta and the rest of Canada. *Journal of Toxicology and Environmental Health-Part A-Current Issues, 74*(22-24), 1609-1620. doi: 10.1080/15287394.2011.618987

Powell, J. H., Amish, S. J., Haynes, G. D., Luikart, G., and Latch, E. K. (2016). Candidate adaptive genes associated with lineage divergence: Identifying SNPs via next-generation targeted resequencing in mule deer (*Odocoileus hemionus*). *Molecular Ecology Resources, 16*(5), 1165-1172. doi: 10.1111/1755-0998.12572

Price, T., and Bouvier, M. (2002). The evolution of F-1 postzygotic incompatibilities in birds. *Evolution, 56*(10), 2083-2089.

Rhymer, J. M., and Simberloff, D. (1996). Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics, 27*(1), 83-109. doi: 10.1146/annurev.ecolsys.27.1.83

Richards, B. (2018). Distribution of Chronic Wasting Disease in North America. U.S. Geological Survey. Accessed from *https://www.usgs.gov/media/images/distribution-chronic-wasting-disease-north-america-0*

Rowe, S., Clarke, S., Van Stijn, T., Hyndman, D., Ward, J., McEwan, K., Dodds, k., … Asher, G. (2015). Developing genomic tools in the new zealand deer industry. *New Zealand Society of Animal Production, 75*, 91-93.

Rusek, J., Ayan, G. B., Turko, P., Tellenbach, C., Giessler, S., Spaak, P., and Wolinska, J. (2015). New possibilities arise for studies of hybridization: SNP-based markers for the multi-species *Daphnia longispina* complex derived from transcriptome data. *Journal of Plankton Research, 37*(3), 626-635. doi: 10.1093/plankt/fbv028

Schwenk, K., Brede, N., and Streit, B. (2008). Introduction. Extent, processes and evolutionary impact of interspecific hybridization in animals. *Philosophical Transactions of the Royal Society B-Biological Sciences, 363*(1505), 2805-2811. doi: 10.1098/rstb.2008.0055

Seehausen, O. (2004). Hybridization and adaptive radiation. *Trends in Ecology and Evolution, 19*(4), 198-207. doi: 10.1016/j.tree.2004.01.003

Stelfox, B. J., and Adamczewski, J. (1993). *Hoofed mammals of Alberta* (2nd ed.) Edmonton, Alberta, Canada: Lone Pine Publishing.

Stephens, M., Clipperton, N., and Bernie, M. (2009). Subspecies-informative SNP assays for evaluating introgression between native golden trout and introduced rainbow trout. *Molecular Ecology Resources, 9*(1), 339-343. doi: 10.1111/j.1755-0998.2008.02407.x

Tokarska, M., Marshall, T., Kowalczyk, R., Wójcik, J. M., Pertoldi, C., Kristensen, T. N., Loeschcke, V., … Bendizen, C. (2009). Effectiveness of microsatellite and SNP markers for parentage and identity analysis in species with low genetic diversity: The case of European bison. *Heredity, 103*, 326. doi: 10.1038/hdy.2009.73

Twyford, A. D., and Ennos, R. A. (2011). Next-generation hybridization and introgression. *Heredity, 108*, 179. doi: 10.1038/hdy.2011.68

Vähä, J., and Primmer, C. (2006). Efficiency of model-based bayesian methods for detecting hybrid individuals under different hybridization scenarios and with different numbers of loci. *Molecular Ecology, 15*(1), 63-72. doi: 10.1111/j.1365-294X.2005.02773.x

Vignal, A., Milan, D., SanCristobal, M., and Eggen, A. (2002). A review on SNP and other types of molecular markers and their use in animal genetics. *Genetics, Selection, Evolution : GSE, 34*(3), 275-305. doi: 10.1186/1297-9686-34-3-275

Wiley, C., Qvarnström, A., Andersson, G., Borge, T., and Saetre, G. (2009). Postzygotic isolation over multiple generations of hybrid descendants in a natural hybrid zone: How well do single-generation estimates reflect reproductive isolation? *Evolution, 63*(7), 1731-1739. doi: 10.1111/j.1558-5646.2009.00674.x

Williams, E. S., Miller, M. W., Kreeger, T. J., Kahn, R. H., and Thorne, E. T. (2002). Chronic wasting disease of deer and elk: A review with recommendations for management. *The Journal of Wildlife Management, 66*(3), 551-563. doi: 10.2307/3803123

Williams, E. (2005). Chronic wasting disease. *Veterinary Pathology, 42*(5), 530-549. doi: 10.1354/vp.42-5-530

Wilson, G., Strobeck, C., Wu, L., and Coffin, J. (1997). Characterization of microsatellite loci in caribou *Rangifer tarandus*, and their use in other artiodactyls. *Molecular Ecology, 6*(7), 697-699. doi: 10.1046/j.1365-294X.1997.00237.x

Wilson, G. A., Nakada, S. M., Bollinger, T. K., Pybus, M. J., Merrill, E. H., and Coltman, D. W. (2009). Polymorphisms at the *PRNP* gene influence susceptibility to chronic wasting disease in two species of deer (*Odocoileus spp.*) in Western Canada. *Journal of Toxicology and Environmental Health-Part A-Current Issues, 72*(17-18), 1025-1029. doi: 10.1080/15287390903084264

Wishart, W. D. (1980). Hybrids of white-tailed and mule deer in Alberta. *Journal of Mammalogy, 61*(4), 716-719. doi: 10.2307/1380318

Wishart, W. D., Hrudka, F., Schmutz, S. M., and Flood, P. F. (1988). Observations on spermatogenesis, sperm phenotype, and fertility in white-tailed X mule deer hybrids and a yak X cow hybrid. *Canadian Journal of Zoology, 66*(7), 1664-1671. doi: 10.1139/z88-240

Wolf, D., Takebayashi, N., and Rieseberg, L. (2001). Predicting the risk of extinction through hybridization. *Conservation Biology, 15*(4), 1039-1053. doi: 10.1046/j.1523-1739.2001.0150041039.x

Wringe, B. F., Stanley, R. R. E., Jeffery, N. W., Anderson, E. C., and Bradbury, I. R. (2017). hybriddetective: A workflow and package to facilitate the detection of hybridization using genomic data in r. *Molecular Ecology Resources, 17*(6), 275-284. doi: 10.1111/1755-0998.12704

Chapter 3: Evaluating introgressive hybridization of mule and white-tailed deer in western Canada

Introduction

Initial steps towards divergence and speciation involve the accumulation of reproductive barriers (Kirkpatrick and Ravigné 2002; Orr and Turelli 2001). Even in the presence of gene flow, given enough time, prezygotic barriers between taxa can accrue via exogenous factors (niche divergence, sexual selection, behavioural isolation, etc.) or strong genetic drift, and eventually give rise to postzygotic, ecologically-independent isolation (Hewitt 2001; Nosil 2008; Abbott et al. 2013; Wang and Bradburd 2014). Both the building up and tearing down of these isolating mechanisms have been topics of contention among historical and contemporary evolutionary biologists. The blurring of genetic boundaries between divergent taxa, especially at the species level, is called hybridization and can give rise to a wide variety of outcomes. These include progressive evolutionary events such as novelty, disruptive selection, and speciation (Dowling and Secor 1997; Seehausen 2004; Lamer *et al.* 2015) but may also have consequences that interfere with management and conservation efforts by compromising coadapted gene complexes, morphological discernment, local adaptation, and the genetic integrity of unique phylogenetic lineages (Leary et al. 1996; Rhymer and Simberloff 1996; Edmands et al. 2009; Martinsen et al. 2001).

Hybridization on a per-individual basis is, by definition, a rare occurrence. Otherwise, it would overcome the products of divergent evolution that act as separating forces to keep sympatric species distinct. But this is not to say that it is uncommon on a per-species basis;

Mallet (2005) estimated that 10% of all animal species and 25% of plant species hybridize. Featured most prominently among hybridizing species are those that are closely related and sympatric in adaptive radiations (Price and Bouvier 2002; Gourbière and Mallet 2010; Abbott et al. 2013) such as mule deer (*Odocoileus hemionus*) (MD) and white-tailed deer (*O. virginianus*) (WT) in western North America (Cronin 1991; Stelfox and Adamczewski 1993; Hornbeck and Mahoney 2000; Bradely et al. 2003). Cervids hold positions of great significance in ecological, economic, and cultural contexts in Canada and the United States. Americans spent nearly $15 billion on domestic big game hunting in 2016 and Canadian expenditures exceeded $1 billion in 2014 (U.S. Fish and Wildlife Service 2016; Federal, Provincial, and Territorial governments of Canada 2014). Although both mule and white-tailed deer are listed as “secure” in Canada and the United States as of 2019, *Odocoileus spp.* and other cervid populations have recently been threatened by chronic wasting disease (CWD), a transmissible spongiform encephalopathy (TSE) akin to mad cow (bovine spongiform encephalopathy) in cattle and Creutzfeldt-Jakob disease in humans (Williams 2005; Miller et al. 2012). CWD is not known to infect humans but poses a serious threat to farmed and wild cervids through its propensity to transmit between individuals and the environment, where the infectious agent can persist for long intervals between hosts (Williams et al. 2002; Miller et al. 2004; Cullingham et al. 2011). The capacity to hybridize adds complexity to an ecologically, economically, and culturally high-leverage system: interspecific hybrids may complicate management policies that regulate the hunting industry and have unknown consequences for the spread of CWD within and between species.

Historically, one of the most essential requisite components in the study of hybrid populations has also been its most problematic roadblock: the ability to reliably identify hybridized individuals. Attempts to catalogue hybrid offspring using morphological markers alone tend to be impractical for a few reasons: offspring physiologies may not be consistently intermediate representations of their heterospecific parents (Leary et al. 1993; Rhymer and Simberloff 1996) and they often display increased phenotypic variation among individuals because any disparity in recombination will affect their genetic makeup more than in their pure species counterparts (Wishart 1980; Harrison and Larson 2014). When morphological data is available for the study of potentially hybridized populations, it is best used in conjunction with genetic data. Furthermore, since introgression of alleles originating from a different taxon may be distributed unevenly throughout the genome, loci should be numerous with highly differentiated allele frequencies between species (Randi et al. 2014; Muirhead and Presgraves 2016). Investigation of the MD x WT hybrid zone in North America contributed insights into past admixture with black-tailed deer (*O.h.columbianus*) (Carr and Hughes 1993; Latch et al. 2011) and adherence to Haldane's rule (Wishart et al. 1988; Hornbeck and Mahoney 2000), but has not yet been examined through the lens of a genetic resource tailored specifically to the molecular ecology of hybridizing species.

Russell et al. (2019) increased the power of hybrid detection in white-tailed and mule deer by incorporating 40 species-specific single nucleotide polymorphisms (SNPs) into an assay that provides a highly diagnostic measure of hybridity. Interrogating admixture and gene flow between divergent taxa using SNPs is quickly becoming standard practice because of their suitability to the task; high abundance in the genome promotes the

presence and discovery of loci with alleles that are species-specific, or at least highly differentiated between species (Stephens and Clipperton 2009; Wiley et al. 2009; Twyford and Ennos 2011; Cullingham et al. 2013; Lamer et al. 2014). As well, the biallelic nature of SNPs simplifies the discernment of two species by recognizing that loci with fixed differences possess a “species A allele” and a “species B allele”. The heightened species-discriminating capability of this resource helps overcome limitations experienced by previous studies, namely the inability to recognize deeper levels of introgression (McClymont et al. 1982; Carr et al. 1986; Hornbeck and Mahoney 2000, Bradley et al. 2003). Because female F1 MD x WT hybrids sometimes retain fertility, backcross generations and introgression of heterospecific alleles are possible (Wishart et al. 1988). The use of multiple loci granted very limited access to early-generation backcrosses but resolution has remained a limiting factor (Ballinger et al. 1992; Derr 1991). Without a considerably powerful method of detection, post-F1 hybrids often go unnoticed because the proportion of their genome inherited from one species is halved at each backcross generation. This logistic rate of change results in cryptic hybrid landscapes and a forfeiture of data that may be integral to the evaluation of admixed populations. Furthermore, studies that fail to recognize advanced-generation backcrosses also fail to recognize the amount of information they forgo. With these precautions in mind, hybrid detection is a data-intensive process and researchers should work to optimize diagnostic power given the limitations of their resources.

Our goals for this study were to (i) conduct a comprehensive survey of hybrid deer in Alberta and (ii) determine whether CWD status is associated with hybridization frequency. These results will provide valuable insights for policy-makers, conservationists, and

researchers seeking to optimize hunting regulations and investigate CWD epizootiology. We accomplished these objectives using a panel of 40 diagnostic SNPs to genotype individuals selected by a sampling method that accounted for CWD status, sex, and geographic location. We then genetically evaluated hybridity using *NewHybrids* version 1.1 beta (Anderson and Thompson 2002) and the R package *introgress* (Buerkle 2005; Gompert and Buerkle 2010).

Methods

Sampling

All deer samples subjected to SNP genotyping were collected from hunter harvested animals that were submitted to the mandatory CWD surveillance program in Alberta, Canada, which monitors the spread and prevalence of the disease. These samples were partitioned using four different grouping strategies: a demographic-matching group, a disease-matching group, a foothills group, and an unidentified species group. Because the deer samples were submitted by hunters, we employed these methods to minimize ascertainment bias caused by uneven distribution of sampling locations, sex, species, and CWD status. Note that species identification during collection was provided by the hunter contributing the sample and recorded by Alberta Fish and Wildlife staff.

The purpose of the demographic-matching group was to randomly sample deer from a large pool while ensuring coverage of the study area and controlling for sex and species. For this approach, the study region (roughly the southern half of Alberta; fig.3) was divided into a grid of 9x17 cells of equal size, 101 of which contained at least one sample of each sex of each species (two concessions were made due to lack of female WT, see table 3). From

those 101 cells, we randomly selected one of each of the following: male MD, female MD, male WT, and female WT. Thus, each sample was one of four different demographic categories from approximately the same geographic location (n=404).

The disease-matching group was designed with a similar pairing-up procedure. We used 250 CWD-positive deer. For each of these individuals, we randomly sampled one same-sex conspecific CWD-negative from the same wildlife management unit. This resulted in an uninfected neighbour whose sex and species was matched to each CWD+ deer (n=500). By controlling for sex, species, and geography of our pool of samples, we could objectively test whether disease status and hybridization rate held any association via Fisher's exact test carried out in R (R core team, 2019). All random sampling for the disease- and demographic-matching groups was performed using R statistical software and the base functions therein.

The foothills group consisted of n=70 samples from western Alberta, near the Rocky Mountains, that were not filtered in any way. This region represents the convergence of alpine and prairie habitats. We theorized that interspecific mating events may be increased in areas where the flat, gentle terrain preferred by WT meets the rugged, irregular terrain frequented by MD (Lingle 2002).

The unidentified species group consisted of those individuals whose species could not be confidently discerned, of which there were n=16. This could potentially be the case if the hunter was uncertain or incorrect in identifying the species, i.e. if the individual was morphologically intermediate. An additional group of *a priori* hybrids from a long term captive-breeding study were included (n=73) (Wishart et al. 1988). Because the parentage of

these individuals is known, we can use the results of their hybrid assignments to further validate the assay and interpret the results of empirical samples.

All samples described above were genotyped on the 40-loci species-discriminating SNP assay developed by Russell et al. (2019). Additionally, we included data from 4996 samples genotyped at 10 microsatellite loci from previous studies. This data set was generated by Cullingham et al. (2010; 2011) and, although it has less collective diagnostic power than the SNP assay, the volume of the data will serve as quantitative support by supplementing the sample size. For sampling, extraction, and genotyping procedures, see Cullingham et al. (2010; 2011).

Extraction and genotyping

All DNA was extracted using a Qiagen 96 DNeasy Blood and Tissue kit following the manufacturer's instructions (Qiagen, Mississauga, Ontario, Canada) and eluted into 150µL of elution buffer. Reactions were performed in 96-well plates. DNA was tested for quantity using a Nanodrop 2000 spectrophotometer. Further DNA quantification using a QuantiFluor assay and Sequenom SNP genotyping procedures were carried out by Neogen Genomics (Neogen Genomics, Lincoln, Nebraska) (Gabriel et al. 2009).

Admixture analysis

We ran simulations to determine which computational methods of hybrid assignment performed well with our data sets. We recognize that *in silico* evaluations do not perfectly replicate *in situ* populations because MD and WT have been sympatric over much of their range in Alberta—and have likely shared at least some gene flow—for many generations. Briefly, 10 MD and 10 WT from outside regions of species overlap (see Russell et al. 2019)

were used as the initial P1 generation to produce two populations containing three backcross generations via the hybridize function in the R package *adegenet* (Jombart 2008; Jombart and Ahmed 2011). Each population contained 100 individuals of each of the following generations: pure WT, pure MD, F1, F2, first, second, and third backcrosses of each species for a net sample size of n=2000. This data set was used as the input for a range of hybrid detection programs: *NewHybrids* version 1.1 (Anderson and Thompson 2002), *snapclust* (Beugin et al. 2018), *structure* version 2.3 (using the above 20 pure individuals as reference parental populations; USEPOPINFO=1) (Pritchard et al. 2000), and hybrid index as calculated in the R package *introgress* (again using allopatric individuals as a reference) (Gompert and Buerkle 2010). At three backcross generations, *NewHybrids* results achieved the lowest rates of false negatives (hybrids mistaken as pure species) and false positives (pure species mistaken as hybrids), so we chose it as our default program. *NewHybrids* uses Bayesian model-based clustering and a Markov chain Monte Carlo algorithm to compute the posterior probabilities of assignment of individuals to hybrid classes (Anderson and Thompson 2002). The classes are specified by the user via an input parameter called “genotype frequency classes”. The number of genotype frequency classes will vary with detection power of loci. 10 frequency classes were set for the SNP data, corresponding to the 10 generations of simulated genotypes. Similar simulations were used to evaluate the efficacy of hybrid assignment by the microsatellite loci, this time using only a single backcross generation because of the reduced power. *NewHybrids*, this time using 6 genotype frequency classes, again minimized error rates and was used going forward. All

NewHybrids runs consisted of 100,000 burnin reps followed by 900,000 reps for data collection.

During the above simulations, we noted that *NewHybrids* assignments were especially conservative; they always slightly underestimated hybridization rates and never observed a false positive in a population with three backcross generations. To assess the detection potential of the data set, we tested the same programs on a population *in silico* with one additional backcross generation (a fourth for populations with SNP genotypes and a second for those with microsatellites). This level of introgression caused *NewHybrids* to introduce occasional false positives. To complement the more conservative *NewHybrids* estimates, we propose the use of thresholds on a continuous measure of hybridity as a high-end mark. By observing the performance of various thresholds *in silico*, we can customize the level of introgression required to recognize a genotype as hybridized in an effort to access partial resolution of deeper introgression. This estimate, along with the estimate by *NewHybrids* at 10 genotype frequency classes (6 in the microsatellite genotypes), will result in a range of hybridization rates that may be adjusted to varying levels of leniency. We used the R package *introgress* to calculate our continuous measure, the hybrid index, which quantifies the genetic contribution of hybridizing species as a single statistic where 0 indicates pure WT and 1 indicates pure MD (Buerkle 2005; Gompert and Buerkle 2010). To choose suitable thresholds that maximize resolution of deep introgression without compromising the ability to differentiate pure from non-pure, we identified the most relaxed thresholds that did not produce any false positive hybrid assignments: 0.04-0.96 in the SNP genotypes and 0.15-0.85 for the microsatellite genotypes (fig.4).

Empirical SNP and microsatellite data sets were assigned to hybrid categories in the same manner as the simulated genotypes: we carried out *NewHybrids* analyses using 10 and 6 genotype frequency classes, respectively. Hybrid index was calculated for all empirical samples using the same methods as the simulated populations. SNP genotypes with hybrid index between 0.04 and 0.96 and microsatellite genotypes between 0.15 and 0.85 were considered potential hybrids. Summary statistics for the microsatellite loci were calculated in the R package *hierfstat* (Goudet 2005) (table 2).

Results

Genotyping

Three samples from the disease-matching group failed genotyping, leaving 987 hunter-submitted samples with call rates averaging 99.82%. Sampling groups break down as follows: 404 demographic-matching, 497 disease-matching (248 CWD- and 249 CWD+), 70 foothills, 16 unidentified-species, and 73 *a priori* hybrids with known pedigrees, plus 4996 individuals previously genotyped at 10 microsatellite loci. See fig.3 for sampling locations and table 3 for a summary of sample group membership.

Simulated admixture analysis

Simulated hybrid populations were used to gauge the efficacy of detection of various levels of introgression by different computational methods when paired with our specific data sets. Each population was independently simulated twice with generations consisting of 100 individuals. Populations were later pooled so that each generation contained n=200 individuals to simplify analyses and reporting of results. Here, two statistics defined in Vähä

and Primmer (2006) are useful to evaluate hybrid assignment: efficiency refers to the proportion of individuals correctly assigned (correct assignments / total individuals of a particular class) and accuracy refers to the proportion of assignments that are correct (correct assignments / total assignments of a particular class).

Hybrid identification was most successful for individuals genotyped at 40 species-discriminating SNP loci. For a population comprised of 10 genotype frequency classes (including pure white-tailed, pure mule deer, F1, F2, and three backcrosses to each species), *NewHybrids* achieved a hybrid accuracy of 1.0 with zero false positives (pure species mistaken as hybrids) and correctly assigned 1578 of 1600 hybrids to a general hybrid class for a combined hybrid efficiency of 0.9863; all hybrid assignments were correct and 98.63% of hybrids were assigned as such. Predictably, the class with the lowest accuracy was the deepest level of introgression, third backcross MD. The lowest efficiency class was second backcross MD. For a population with 12 genotype frequency classes, including the 10 listed above plus a fourth backcross, hybrid index with thresholds at 0.04 and 0.96 produced a hybrid accuracy of 1.0 (zero false positives) and efficiency of 0.79. Widening the thresholds introduced false positives and tightening decreased efficiency (fig.4). Note that this method does not recognize individual generations but describes a general distinction between hybrid and pure species. As well, *structure* Q values were nearly identical to hybrid index (r^2 = 0.999) when used with reference populations (USEPOPINFO = 1); for our purposes, these two methods were interchangeable.

Hybrid resolution was diminished in the microsatellite data set. In a population with 6 genotype frequency classes (including a single backcross generation), *NewHybrids* analysis

resulted in a combined hybrid accuracy of ~1.0 (1 of 400 pure species was assigned as a hybrid) and efficiency of 0.99. Hybrid index thresholds were again chosen as the point of maximum efficiency without committing a false positive and were more strict than in the SNP populations. Thresholds at 0.15 and 0.85, when applied to a simulated population with 8 genotype frequency classes, identified hybrids with an accuracy of 1.0 and efficiency of 0.78.

Empirical admixture analysis

Of the 987 samples genotyped at diagnostic SNPs, 3 were identified as hybrids by *NewHybrids*, for a hybridization rate of 0.30%. They included a first backcross MD from the demographic-matching group, a third backcross MD that tested positive for CWD, and a third backcross WT from the unidentified species group. These 3 individuals, plus 8 more, had hybrid indexes between 0.04 and 0.96 (1.12% of the total). These 8 additional deer included 4 from the demographic-matching group (none from the same cell), 2 female WT from the foothills of the Rockies, and 2 whose species could not be identified. Fisher's exact test indicated no association between CWD status and hybrid status ($p = 0.96$). The deepest level of introgression of the *a priori* hybrids was the 3rd backcross generation; all were assigned correctly as hybrids.

In 4996 individuals genotyped at microsatellite loci, 3 were identified as hybrids by *NewHybrids* for a rate of 0.06%. Two were F2s and one was a backcross MD. 30 more had hybrid indexes between 0.15 and 0.85 (0.66% of the total). See table 2 for microsatellite summary statistics. Hybrids from both data sets do not appear to be associated with any geographic factors. See figure 4 for sampling locations and hybrid occurrences.

Discussion

Rates of hybridization were evaluated in a putative hybrid zone of mule and white-tailed deer. Here, we analyzed a sympatric population with conservative and relaxed introgression estimators in tandem to capture low and high ends of a range of hybridization rates, respectively. The 987 deer genotyped at species-discriminating SNPs included 3 to 11 hybrid individuals and the 4996 deer genotyped at microsatellite loci contained 3 to 33 hybrids. The highest rate of hybridization was observed by estimating the hybrid index of those individuals with SNP genotypes, that is, the samples submitted by hunters as part of a CWD monitoring program. Hybridization appears slightly more common in this sample group because it offers increased power to detect lower levels of introgression but estimates were still modest at 0.3 to 1.1%. Given the small hybrid sample size, we will not attempt to draw conclusions about the greater hybrid zone or its ecological basis from the geographic patterns of occurrences observed here. No F1s were identified in this study; all hybrids encountered were post-F1 backcrosses. The most frequent hybrid categories were also the deepest levels of introgression detectable by each molecular assay: third backcross—with partial resolution of a fourth—by the SNP loci and first backcross—with partial resolution of a second—by the microsatellites. Furthermore, many individuals, especially from the empirical SNP collection, that did not meet the threshold requirement to be classified as hybrids still had hybrid indexes between 0 and 1. This suggests either widespread, prolonged gene flow or very low levels of incomplete lineage sorting (Zhou et al. 2016). The two are difficult to distinguish: we've concluded here that contemporary gene flow does occur and the species' history of sympatry implies that it has for a long time but ancestral polymorphisms can sometimes be

retained in recently diverged species and persist at low frequencies as rare alleles (Hudson and Coyne 2002). The skewed distribution of classes identified by *NewHybrids* towards deeper introgression levels suggests that those individuals with insignificant but non-terminal hybrid indexes may have some cryptic hybrid ancestry. To quantify that minute genetic ancestry would require high-throughput, possibly genomic-level, data (Gompert et al. 2017).

This bimodality suggests considerable underdominance and aligns with previous studies. Lingle (1993) found evidence that early-generation hybrids may suffer reduced fitness from biomechanically inefficient escape gaits. Wishart et al. (1988) observed increased maturation and quality of spematozoa in later-generation hybrids compared to F1s and concluded that the MD x WT hybrid system adheres strictly to Haldane's rule, which predicts that heterozygote breakdown will affect the heterogametic sex more adversely than the homogametic sex (Haldane 1922). Hornbeck and Mahoney (2000) also reported "maladaptive behaviour in a number of respects that would indicate reduced fitness". This study contributes to the mounting evidence that hybrids are generally less fit than their pure species parents and that fitness increases with each backcross generation. If this is true, the system may be said to be a tension zone where hybrid populations are maintained by a balance of migration and selection against hybrids, with the strongest selective pressure acting on early-generation hybrids (Hewitt 1988; Hu 2005). The low frequencies of hybridization observed here are consistent with this theory. Similar low rates have been reported previously: 1-2% in southwestern U.S. (Derr 1991), 2% in Montana (Cronin et al. 1988), and 3-4% in Alberta (Hughes and Carr 1993). Rates as high as 6% have been observed in Alberta (Hornbeck and Mahoney 2000) but, in that instance, all hybrids were

found in one location where human-mediated environmental disturbance may have fostered increased hybridization (Todesco et al 2016). Similar hybrid hotspots were confirmed in the comparable sika (*Cervus nippon*) x red deer (*C. elaphus*) hybrid system in the U.K., where both species experience little predation pressure (Senn and Pemberton 2009; Senn et al. 2010). Localized hotspots are characteristic of mosaic hybrid zones: areas of species overlap with a patchy distribution of some ecological factor that favours one species over the other (Bierne et al. 2002; Ross and Harrison 2002; M'Gonigle and FitzJohn 2010; Bierne et al. 2013).

Only one hybrid tested positive for CWD. This does not exceed expectations based on chance given the hybridization rates and number of CWD+ individuals sampled (Fisher's exact test: $p>0.05$). Hybridization events may, in theory, foster the spread of CWD when they do occur but because hybrids are so infrequent, they likely play a diminished role. We also recognize that, depending on reproductive success rates, hybrid sample size may be a suitable proxy but will underestimate interspecific mating attempts (Wishart et al. 1988). Because CWD infectious particles transmit laterally among individuals in close proximity, the frequency of mating attempts—successful or not—may affect overall disease expansion rates (Miller et al. 2000; Williams 2005; Cullingham et al. 2010; 2011; 2012; Nalls et al. 2013). Further research on the propensity of heterospecifics to attempt mating will help resolve this question.

Wild populations of Alberta have low hybridization rates but rates may vary in other environments. Captive populations such as cervid farms are unlikely to stock both species in the same enclosure but farmed animals can sometimes contribute to local allele frequencies

and gene flow, usually via escapes from the facility (Russo et al. 2019). Populations in other parts of the hybrid zone may vary as well. MD and WT are sympatric throughout much of western North America. The ecological nuances of different habitats can cause hybrid zones to produce a variety of outcomes, even between multiple instances of the same parental species (Gompert and Buerkle 2016). For wildlife managers and researchers interested in areas of this hybrid zone outside of Alberta, use of the resources and methods reported here may draw different results.

Although the distribution of hybrid classes may be informative of contemporary trends, for our purposes, the distinction between hybrid and non-hybrid was more important than the discernment of any individual hybrid class. This was one reason we chose to complement the discrete, generational assignments of *NewHybrids* with the continuous scale of hybrid index. Gompert and Beurkle (2016) warn that the use of discrete classes may obscure variation within categories, neglecting individuality that may be helpful to handle conservation on a case-by-case basis. We should note here that the slightly decreased assignment efficiency of the simulated second backcross generation in the SNP data set was most likely caused by its allele frequencies resembling neighbouring hybrid classes more than any other. As is the case, any missed second backcrosses were misassigned to another hybrid class.

We have shown here that the MD and WT of Alberta hybridize at a similar, perhaps slightly lower rate than that reported in other parts of the hybrid zone and in other comparable systems (Cronin et al. 1988; Derr 1991; Hughes and Carr 1993; Senn and Pemberton 2009; Senn et al. 2010). Interspecific reproductive barriers of these species are

semipermeable but hybrids are still a rare occurrence. When a hybridization event does take place, the resulting offspring is likely the target of more intense selective pressure than that applied to its backcrossed descendants and pure species peers. Phylogenetic lineages in Alberta appear to remain mostly intact and well-structured.

Table 2. Microsatellite summary statistics. Overall row (bottom) contains total allele counts (Na), observed heterozygosities (Ho) and gene diversities (Hs) when species are combined, as well as interspecific Fst and corrected Fstp averaged across loci as calculated by *hierfstat*.

Locus name	N_a	N_a MD	N_a WT	H_o MD	H_o WT	H_s MD	H_s WT	Fst	Fstp
BBJ2	12	9	8	0.7703	0.5136	0.7757	0.5247	0.2088	0.3454
BM6438	16	10	16	0.6022	0.8722	0.6063	0.8846	0.1273	0.2259
INRA011	12	6	9	0.1556	0.5017	0.1591	0.5067	0.499	0.6657
N	30	14	28	0.8398	0.8325	0.8522	0.8919	0.0339	0.0656
BL25	11	8	7	0.6732	0.4519	0.6882	0.4905	0.2565	0.4082
BM4107	20	17	17	0.6398	0.7599	0.6674	0.8115	0.1405	0.2462
Eth152	19	13	18	0.8604	0.6045	0.8691	0.7628	0.0751	0.1397
K	9	7	6	0.6538	0.3701	0.6604	0.3774	0.2809	0.4386
Rt5	23	10	18	0.7679	0.821	0.7735	0.8255	0.1091	0.1967
Rt7	18	13	15	0.7721	0.8446	0.7842	0.8546	0.0511	0.0972
Overall	170	107	142	0.6654		0.6883		0.1668	0.2858

Table 3. Breakdown of sample group membership. The sexes of one CWD+ mule deer[1] and one individual of unidentified species[2] were unknown but are included in the totals (far right column).

Sampling group	MD males	MD females	WT males	WT females	Total males	Total females	Total MD	Total WT	CWD+	CWD-	Total n
Demographic-matching	102	99	101	102	203	201	201	203	0	404	404
Disease-matching[1]	326	117	39	14	365	131	444	53	249	248	497
Foothills	11	7	23	29	34	36	18	52	0	70	70
Unidentified species[2]	-	-	-	-	7	8	-	-	0	16	16
Microsatellite group	-	-	-	-	-	-	2914	2082	-	-	4996

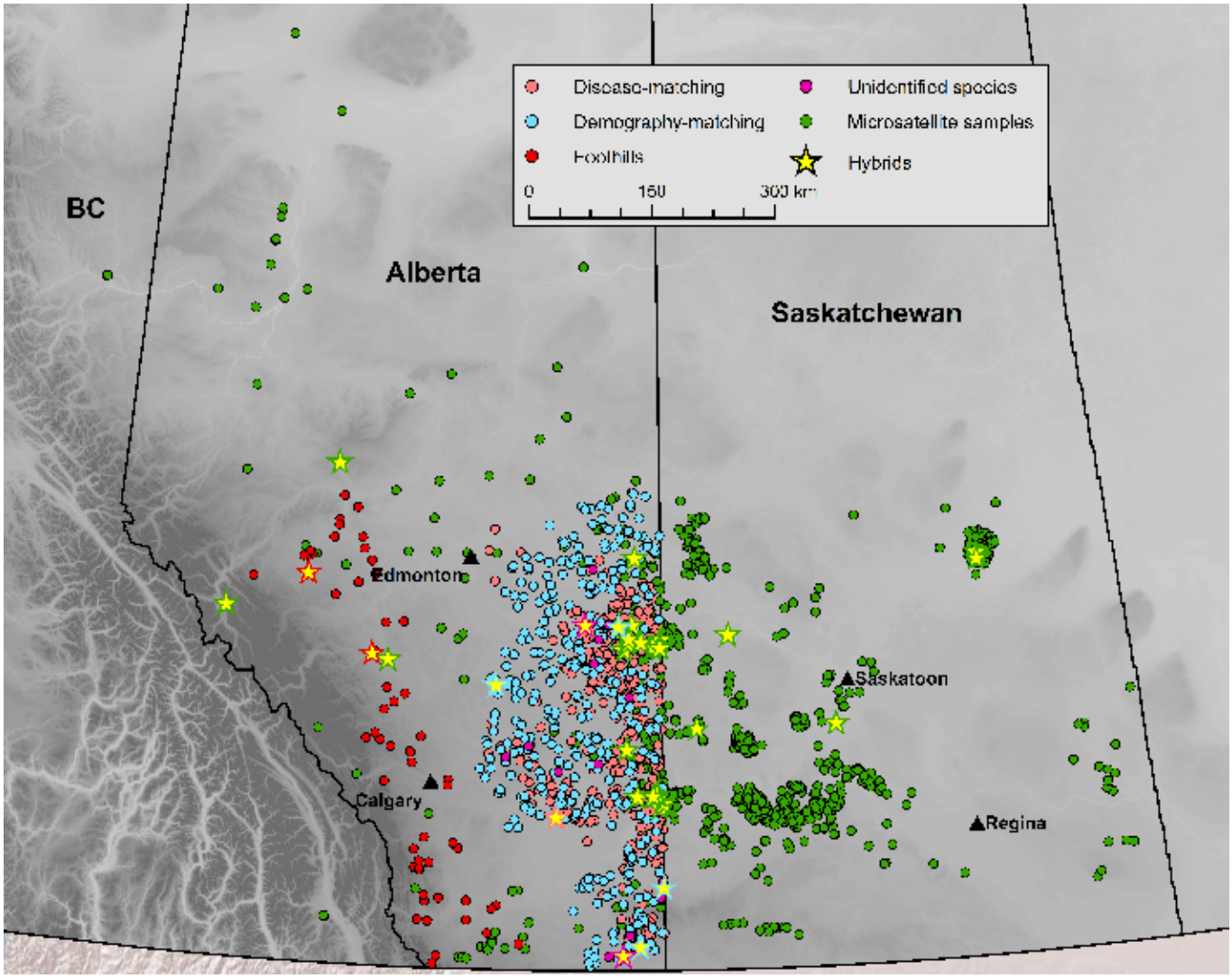

Figure 3. Empirical samples for admixture analysis. All samples genotyped at SNP loci were from Alberta, some in the microsatellite data set were from BC and Saskatchewan. Outline colour of the hybrid symbol indicates the sampling group to which it belongs.

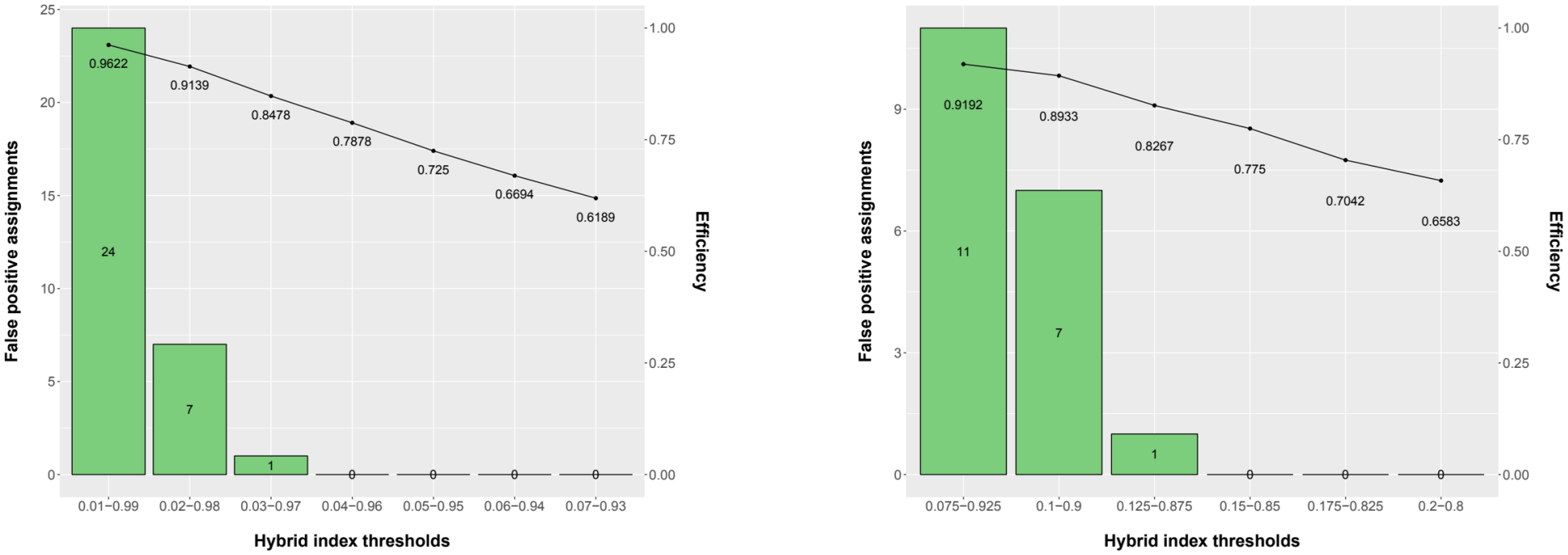

Figure 4. False positive assignments (pure mistaken as hybrid; represented by bars) and assignment efficiency (hybrids correctly assigned / total hybrids; represented by line) at different hybrid index thresholds. We chose the most relaxed thresholds that did not produce any false positives: 0.04-0.96 for SNP genotypes (a) and 0.15-0.85 for microsatellite genotypes (b).

References

Abbott, R., Albach, D., Ansell, S., Arntzen, J. W., Baird, S. J. E., Bierne, N., . . . Zinner, D. (2013). Hybridization and speciation. *Journal of Evolutionary Biology, 26*(2), 229-246. doi:10.1111/j.1420-9101.2012.02599.x

Anderson, E. C., & Thompson, E. A. (2002). A model-based method for identifying species hybrids using multilocus genetic data. *Genetics, 160*(3), 1217. Retrieved from http://www.genetics.org/content/160/3/1217.abstract

Ba, H., Li, Z., Yang, Y., & Li, C. (2018). Development of diagnostic SNP markers to monitor hybridization between sika deer (cervus nippon) and wapiti (cervus elaphus). *G3-Genes Genomes Genetics, 8*(7), 2173-2179. doi:10.1534/g3.118.200417

Ballinger, S., Blankenship, L., Bickham, J., & Carr, S. (1992). Allozyme and mitochondrial DNA analysis of a hybrid zone between white-tailed deer and mule deer (odocoileus) in west texas. *Biochemical Genetics, 30*, 1-11. doi:10.1007/BF00554423

Beugin, M., Gayet, T., Pontier, D., Devillard, S., & Jombart, T. (2018). A fast likelihood solution to the genetic clustering problem. *Methods in Ecology and Evolution, 9*(4), 1006-1016. doi:10.1111/2041-210X.12968

Bierne, N., David, P., Langlade, A., & Bonhomme, F. (2002). Can habitat specialisation maintain a mosaic hybrid zone in marine bivalves? *Marine Ecology Progress Series, 245*, 157-170. doi:10.3354/meps245157

Bierne, N., Gagnaire, P., & David, P. (2013). The geography of introgression in a patchy environment and the thorn in the side of ecological speciation. *Current Zoology, 59*(1), 72-86. doi:10.1093/czoolo/59.1.72

Bradley, R. D., Bryant, F. C., Bradley, L. C., Haynie, M. L., Baker, R. J., & Jones, C. A. (2003). Implications of hybridization between white-tailed deer and mule deer. *The Southwestern Naturalist, 48*(4), 654-660. doi:10.1894/0038-4909(2003)048<0654:IOHBWD>2.0.CO;2

Buerkle, C. A. (2005). Maximum-likelihood estimation of a hybrid index based on molecular markers. *Molecular Ecology Notes, 5*(3), 684-687. doi:10.1111/j.1471-8286.2005.01011.x

Carr, S. M., Ballinger, S. W., Derr, J. N., Blankenship, L. H., & Bickham, J. W. (1986). Mitochondrial DNA analysis of hybridization between sympatric white-tailed deer and mule deer in west texas. *Proc Natl Acad Sci USA, 83*(24), 9576. Doi: 10.1073/pnas.83.24.9576

Carr, S., & Hughes, G. (1993). Direction of introgressive hybridization between species of north-american deer (odocoileus) as inferred from mitochondrial-cytochrome-B sequences.*Journal of Mammalogy, 74*(2), 331-342. doi:10.2307/1382388

Cronin, M. A. (1991). Mitochondrial and nuclear genetic relationships of deer (odocoileus spp.) in western north america. *Canadian Journal of Zoology, 69*(5), 1270-1279. doi:10.1139/z91-179

Cronin, M. A., Vyse, E. R., & Cameron, D. G. (1988). Genetic relationships between mule deer and white-tailed deer in montana. *The Journal of Wildlife Management, 52*(2), 320-328. doi:10.2307/3801243

Cullingham, C. I., Cooke, J. E., K., D., S., & Coltman, D. W. (2013). A species-diagnostic SNP panel for discriminating lodgepole pine, jack pine, and their interspecific hybrids. *Tree Genetics & Genomes, 9*(4), 1119-1127. doi: 10.1007/s11295-013-0608-x

Cullingham, C. I., Nakada, S. M., Merrill, E. H., Bollinger, T. K., Pybus, M. J., & Coltman, D. W. (2011). Multiscale population genetic analysis of mule deer (odocoileus hemionus hemionus) in western canada sheds new light on the spread of chronic wasting disease. *Canadian Journal of Zoology, 89*(2), 134-147. doi:10.1139/Z10-104

Cullingham, C. I., Merrill, E. H., Pybus, M. J., Bollinger, T. K., Wilson, G. A., & Coltman, D. W. (2010). Broad and fine-scale genetic analysis of white-tailed deer populations: Estimating the relative risk of chronic wasting disease spread. *Evolutionary Applications, 4*(1), 116-131. doi:10.1111/j.1752-4571.2010.00142.x

Derr, J. (1991). Genetic interactions between white-tailed and mule deer in the southwestern united states. *The Journal of Wildlife Management, 55*, 228. doi:10.2307/3809144

Dowling, T., & Secor, C. (1997). The role of hybridization and introgression in the diversification of animals. *Annual Review of Ecology and Systematics, 28*, 593-619. doi:10.1146/annurev.ecolsys.28.1.593

Edmands, S., Northrup, S. L., & Hwang, A. S. (2009). Maladapted gene complexes within populations of the intertidal copepod *tigriopus californicus*? *Evolution, 63*(8), 2184-2192. doi:10.1111/j.1558-5646.2009.00689.x

Gabriel, S., Ziaugra, L., & Tabbaa, D. (2009). SNP genotyping using the sequenom MassARRAY iPLEX platform. *Current Protocols in Human Genetics, 60*(1), 2.12. 1-2.12. 18.

Gompert, Z., & Buerkle, C. A. (2016). What, if anything, are hybrids: Enduring truths and challenges associated with population structure and gene flow. *Evolutionary Applications, 9*(7), 909-923. doi:10.1111/eva.12380

Gompert, Z., & Buerkle, C. A. (2010). INTROGRESS: A software package for mapping components of isolation in hybrids. *Molecular Ecology Resources, 10*(2), 378-384. doi:10.1111/j.1755-0998.2009.02733.x

Gompert, Z., Mandeville, E. G., & Buerkle, C. A. (2017). Analysis of population genomic data from hybrid zones. *Annual Review of Ecology, Evolution, and Systematics, 48*(1), 207-229. doi:10.1146/annurev-ecolsys-110316-022652

Goudet, J. (2005). Hierfstat, a package for r to compute and test hierarchical F-statistics. *Molecular Ecology Notes, 5*(1), 184-186. doi:10.1111/j.1471-8286.2004.00828.x

Gourbière, S., & Mallet, J. (2010). Are species real? the shape of the species boundary with exponential failure, reinforcement, and the "missing snowball". *Evolution, 64*(1), 1-24. doi:10.1111/j.1558-5646.2009.00844.x

Haldane, J. (1922). Sex ratio and unisexual sterility in hybrid animals. *Journal of Genetics, 12*(2), 101-109. doi:10.1007/BF02983075

Harrison, R. G., & Larson, E. L. (2014). Hybridization, introgression, and the nature of species boundaries. *Journal of Heredity, 105*(S1), 795-809. doi:10.1093/jhered/esu033

Hewitt, G. M. (1988). Hybrid zones-natural laboratories for evolutionary studies. *Trends in Ecology & Evolution, 3*(7), 158-167. doi:10.1016/0169-5347(88)90033-X

Hewitt, G. M. (2001). Speciation, hybrid zones and phylogeography — or seeing genes in space and time. *Molecular Ecology, 10*(3), 537-549. doi:10.1046/j.1365-294x.2001.01202.x

Hornbeck, G. E., & Mahoney, J. M. (2000). Introgressive hybridization of mule deer and white-tailed deer in southwestern alberta. *Wildlife Society Bulletin (1973-2006), 28*(4), 1012-1015. doi: 10.2307/3783860

Hu, X. (2005). Tension versus ecological zones in a two-locus system. *Theoretical Population Biology, 68*(2), 119-131. doi:10.1016/j.tpb.2005.02.003

Hudson, R. R., & Coyne, J. A. (2002). Mathematical consequences of the genealogical species concept. *Evolution, 56*(8), 1557-1565. doi:10.1111/j.0014-3820.2002.tb01467.x

Hughes, G. A., & Carr, S. M. (1993). Reciprocal hybridization between white-tailed deer (odocoileus virginianus) and mule deer (O. hemionus) in western canada: Evidence

from serum albumin and mtDNA sequences. *Canadian Journal of Zoology, 71*(3), 524-530. doi:10.1139/z93-073

Jombart, T. (2008). Adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics, 24*(11), 1403-1405. doi:10.1093/bioinformatics/btn129

Jombart, T., & Ahmed, I. (2011). Adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. *Bioinformatics, 27*(21), 3070-3071. doi:10.1093/bioinformatics/btr521

Kirkpatrick, M., & Ravigné, V. (2002). Speciation by natural and sexual selection: Models and experiments. *The American Naturalist, 159*, S22-S35. doi:10.1086/338370

Lamer, J. T., Ruebush, B. C., Arbieva, Z. H., McClelland, M. A., Epifanio, J. M., & Sass, G. G. (2015). Diagnostic SNPs reveal widespread introgressive hybridization between introduced bighead and silver carp in the mississippi river basin. *Molecular Ecology, 24*(15), 3931-3943. doi:10.1111/mec.13285

Lamer, J. T., Sass, G. G., Boone, J. Q., Arbieva, Z. H., Green, S. J., & Epifanio, J. M. (2014). Restriction site-associated DNA sequencing generates high-quality single nucleotide polymorphisms for assessing hybridization between bighead and silver carp in the united states and china. *Molecular Ecology Resources, 14*(1), 79-86. doi:10.1111/1755-0998.12152

Latch, E. K., Kierepka, E. M., Heffelfinger, J. R., & Rhodes, O. E. (2011). Hybrid swarm between divergent lineages of mule deer (odocoileus hemionus). *Molecular Ecology, 20*(24), 5265-5279. doi:10.1111/j.1365-294X.2011.05349.x

Leary, R. E., Gould, W. R., & Sage, G. K. (1996). Success of basibranchial teeth in indicating pure populations of rainbow trout and failure to indicate pure populations of westslope cutthroat trout. *North American Journal of Fisheries Management, 16*(1), 210-213. doi:10.1577/1548-8675(1996)016<0210:SOBTII>2.3.CO;2

Lingle, S. (1993). Escape gaits of white-tailed deer, mule deer, and their hybrids: Body configuration, biomechanics, and function. *Canadian Journal of Zoology, 71*(4), 708-724. doi:10.1139/z93-095

Lingle, S. (2002). Coyote predation and habitat segregation of white-tailed deer and mule deer. *Ecology, 83*(7), 2037-2048. doi:10.1890/0012-9658(2002)083[2037:CPAHSO]2.0.CO;2

Mallet, J. (2005). Hybridization as an invasion of the genome. *Trends in Ecology & Evolution, 20*(5), 229-237. doi:10.1016/j.tree.2005.02.010

Martinsen, G., Whitham, T., Turek, R., & Keim, P. (2001). Hybrid populations selectively filter gene introgression between species. *Evolution, 55*(7), 1325-1335. doi:10.1111/j.0014-3820.2001.tb00655.x

McClymont, R., Fenton, M., & Thompson, J. (1982). Identification of cervid tissues and hybridization by serum-albumin. *Journal of Wildlife Management, 46*(2), 540-544. doi:10.2307/3808677

M'Gonigle, L. K., & FitzJohn, R. G. (2010). Assortative mating and spatial structure in hybrid zones. *Evolution, 64*(2), 444-455. doi:10.1111/j.1558-5646.2009.00838.x

Miller, M. W., Wolfe, L. L., Sirochman, T. M., Sirochman, M. A., Jewell, J. E., & Williams, E. S. (2012). Survival patterns in white-tailed and mule deer after oral inoculation with a standardized, conspecific prion dose. *Journal of Wildlife Diseases, 48*(2), 526-529. doi:10.7589/0090-3558-48.2.526

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

Miller, M., Williams, E., McCarty, C., Spraker, T., Kreeger, T., Larsen, C., & Thorne, E. (2000). Epizootiology of chronic wasting disease in free-ranging cervids in colorado and wyoming.*Journal of Wildlife Diseases, 36*(4), 676-690. doi:10.7589/0090-3558-36.4.676

Muirhead, C. A., & Presgraves, D. C. (2016). Hybrid incompatibilities, local adaptation, and the genomic distribution of natural introgression between species. *The American Naturalist, 187*(2), 249-261. doi:10.1086/684583

Nalls, A. V., McNulty, E., Powers, J., Seelig, D. M., Hoover, C., Haley, N. J., . . . Mathiason, C. K. (2013). Mother to offspring transmission of chronic wasting disease in reeves's muntjac deer. *Plos One, 8*(8), e71844. doi: 10.1371/journal.pone.0071844

Nosil, P. (2008). Speciation with gene flow could be common. *Molecular Ecology, 17*(9), 2103-2106. doi:10.1111/j.1365-294X.2008.03715.x

Orr, H., & Turelli, M. (2001). The evolution of postzygotic isolation: Accumulating dobzhansky-muller incompatibilities. *Evolution, 55*(6), 1085-1094. doi:10.1111/j.0014-3820.2001.tb00628.x

Price, T., & Bouvier, M. (2002). The evolution of F-1 postzygotic incompatibilities in birds. *Evolution, 56*(10), 2083-2089. doi:10.1111/j.0014-3820.2002.tb00133.x

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics, 155*(2), 945-959.

Randi, E., Hulva, P., Fabbri, E., Galaverni, M., Galov, A., Kusak, J., . . . Caniglia, R. (2014). Multilocus detection of wolf x dog hybridization in italy, and guidelines for marker selection. *Plos One, 9*(1), e86409. doi:10.1371/journal.pone.0086409

Rhymer, J. M., & Simberloff, D. (1996). Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics, 27*(1), 83-109. doi:10.1146/annurev.ecolsys.27.1.83

Ross, C., & Harrison, R. (2002). A fine-scale spatial analysis of the mosaic hybrid zone between gryllus firmus and gryllus pennsylvanicus. *Evolution, 56*(11), 2296-2312. doi:10.1554/0014-3820(2002)056[2296:AFSSAO]2.0.CO;2

Russell, T., Cullingham, C., Kommadath, A., Stothard, P., Herbst, A., & Coltman, D. (2019). Development of a novel mule deer genomic assembly and species-diagnostic SNP panel for assessing introgression in mule deer, white-tailed deer, and their interspecific hybrids. *G3: Genes|genomes|genetics, 9*(3), 911-919. doi:10.1534/g3.118.200838

Russo, I. M., Hoban, S., Bloomer, P., Kotze, A., Segelbacher, G., Rushworth, I., . . . Bruford, M. W. (2019). "Intentional genetic manipulation' as a conservation threat. *Conservation Genetics Resources, 11*(2), 237-247. doi:10.1007/s12686-018-0983-6

Seehausen, O. (2004). Hybridization and adaptive radiation. *Trends in Ecology & Evolution, 19*(4), 198-207. doi:10.1016/j.tree.2004.01.003

Senn, H. V., & Pemberton, J. M. (2009). Variable extent of hybridization between invasive sika (cervus nippon) and native red deer (C-elaphus) in a small geographical area. *Molecular Ecology, 18*(5), 862-876. doi:10.1111/j.1365-294X.2008.04051.x

Senn, H. V., Swanson, G. M., Goodman, S. J., Barton, N. H., & Pemberton, J. M. (2010). Phenotypic correlates of hybridisation between red and sika deer (genus cervus). *Journal of Animal Ecology, 79*(2), 414-425. doi:10.1111/j.1365-2656.2009.01633.x

Stelfox, B. J., & Adamczewski, J. (1993). *Hoofed mammals of alberta*. Edmonton, Alberta, Canada: Lone Pine Publishing.

Stephens, M., Clipperton, N., & Bernie, M. (2009). Subspecies-informative SNP assays for evaluating introgression between native golden trout and introduced rainbow trout. *Molecular Ecology Resources, 9*(1), 339-343. doi:10.1111/j.1755-0998.2008.02407.x

Todesco, M., Pascual, M. A., Owens, G. L., Ostevik, K. L., Moyers, B. T., Hubner, S., . . . Rieseberg, L. H. (2016). Hybridization and extinction. *Evolutionary Applications, 9*(7), 892-908. doi:10.1111/eva.12367

Twyford, A. D., & Ennos, R. A. (2011). Next-generation hybridization and introgression. *Heredity, 108*, 179. doi:10.1038/hdy.2011.68

Vähä, J., & Primmer, C. (2006). Efficiency of model-based bayesian methods for detecting hybrid individuals under different hybridization scenarios and with different numbers of loci.*Molecular Ecology, 15*(1), 63-72. doi:10.1111/j.1365-294X.2005.02773.x

Wang, I. J., & Bradburd, G. S. (2014). Isolation by environment. *Molecular Ecology, 23*(23), 5649-5662. doi:10.1111/mec.12938

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. Evolution, 38(6), 1358-1370. doi:10.1111/j.1558-5646.1984.tb05657.x

Wiley, C., Qvarnström, A., Andersson, G., Borge, T., & Saetre, G. (2009). Postzygotic isolation over multiple generations of hybrid descendants in a natural hybrid zone: How well do single-generation estimates reflect reproductive isolation? *Evolution, 63*(7), 1731-1739. doi:10.1111/j.1558-5646.2009.00674.x

Williams, E. S., Miller, M. W., Kreeger, T. J., Kahn, R. H., & Thorne, E. T. (2002). Chronic wasting disease of deer and elk: A review with recommendations for management. *The Journal of Wildlife Management, 66*(3), 551-563. doi:10.2307/3803123

Williams, E. (2005). Chronic wasting disease. *Veterinary Pathology, 42*(5), 530-549. doi:10.1354/vp.42-5-530. doi:10.1354%2Fvp.42-5-530

Wishart, W. D. (1980). Hybrids of white-tailed and mule deer in alberta. *Journal of Mammalogy, 61*(4), 716-719. doi:10.2307/1380318

Wishart, W. D., Hrudka, F., Schmutz, S. M., & Flood, P. F. (1988). Observations on spermatogenesis, sperm phenotype, and fertility in white-tailed X mule deer hybrids and a yak X cow hybrid. *Canadian Journal of Zoology, 66*(7), 1664-1671. doi:10.1139/z88-240

Zhou, Y., Duvaux, L., Ren, G., Zhang, L., Savolainen, O., & Liu, J. (2016). Importance of incomplete lineage sorting and introgression in the origin of shared genetic variation between two closely related pines with overlapping distributions. *Heredity, 118*, 211. doi:10.1038/hdy.2016.72

Chapter 4: Conclusions

In this thesis, I've ameliorated the molecular methods previously available to quantify introgression in mule and white-tailed deer by incorporating 40 species-discriminating loci into a SNP assay. This resource improves upon past hybrid detection capabilities and can reliably estimate introgression as deep as that present in third or fourth generation hybrids. I then genotyped 987 deer in a comprehensive survey of the state of hybrid deer in Alberta. I evaluated hybridity using two different computational methods to yield a range of estimates; the conservative and discrete classes of *NewHybrids* together with the customizable and continuous hybrid index of *introgress* offered the most realistic estimate of genome-wide introgression.

Just over 1% of all deer were hybridized. This is a lower figure than I initially expected given previously described rates in Alberta (6% from Hornbeck and Mahoney 2000; 3.6% from Hughes and Carr 1993) and Texas (~20% from Ballinger et al. 1992; 4.7% from Bradley et al. 2003; 5.6% from Stubblefield et al. 1986). Even rates of hybridization similar to my own study that had been previously reported in south-western United States (Derr 1991) and Montana (Cronin et al. 1988) are not equivalent considering they used methods that were less sensitive to backcross generations than ours. This discrepancy can mostly be explained by project design differences and intrinsic biases. Previous studies—many were from the 80s and 90s—were constrained by the molecular throughput limitations of the time. To compensate, they analyzed smaller sample sizes from populations where hybrids had already been observed. Small sample sizes paired with low true rates of hybridization can increase variation in results. For example, in a sample size of n=50, even one or two

hybridized deer can inflate results beyond those found here. Consider also the difficulties one might have in publishing such a study that finds zero hybrids, a very plausible outcome. For this reason, the goals outlined by many previous studies were not necessarily to provide an objective estimate of hybrid pervasiveness, but to characterize hybrids when they did occur and to offer insight as to why they might be present in a particular locale. With the exception of Hughes and Carr (1993; 2 hybrids in n=55 deer), these studies with high hybrid rates tended to sample from populations or small geographic areas based on putative hybrid sightings prior to any molecular analyses. Alternatively, my study has taken a more holistic approach that reflects my attempt to answer a different question, specifically: how common are hybrids among the greater population in a region of species overlap?

Of the 11 hybrids I genotyped at species-discriminating SNPs, all were backcrosses and 10 were second-generation backcrosses or deeper. The lack of F1s indicates severe underdominance, which agrees with previous findings. Signs of underdominance have been a common observation among studies of hybrid deer. In an assessment of escape gait biomechanics, Lingle (1993) found hybrids to display severe inefficiencies in landing and takeoff trajectories as well as inconsistent hoof placement, even in successive strides of the same individual. Overall maladaptive behaviour and traits were also noted by Hornbeck and Mahoney (2000). As well as heterozygote breakdown, the deficiency of F1 hybrids observed in this study indicates the heavily impaired reproductive efficacy of interspecific mating. The difficulty of breeding hybrids in captivity (as in Wishart et al. 1988) suggests that low interspecific reproductive rates are another factor limiting the proliferation of hybrid deer in wild populations. It appears that the production of hybrids, especially in natural settings, is

prevented by an abundance of strong prezygotic barriers—such as visual, auditory, and olfactory cues, habitat segregation, and reduced resource competition—as well as physiological postzygotic barriers—including ineffective escape gaits and decreased quality of spermatozoa (Airst and Lingle 2019; Berry et al. 2019; Wishart et al. 1988; Wood et al. 1989).

Despite these barriers, backcrossed hybrids occur in low numbers in wild populations. Reproductive function has been shown to improve after repeated backcrossing (Wishart et al. 1988), suggesting a propensity for overall fitness to return as the proportion of introgressed genome is reduced. This fitness return should correspond to the amount of Dobzhansky-Muller incompatibilities alleviated by each successive backcross generation (Orr and Turelli 2001). Since thousands of generations of natural selection have optimized the function of MD and WT genomes separately, it follows that hybrid fitness will be negatively correlated with genetic distance from the closest parental genome. Furthermore, because the amount of introgressed genome is halved at each backcross, fitness should return logistically with each generation. Theoretically, the suddenness of fitness increases in backcrosses may be one of only a few aspects favouring the persistence of hybrids. If fitness returns quickly, a hybrid lineage may only have to endure a few generations of underdominance before its pure species faculties are restored. This would explain both the presence of advanced-generation hybrids and the absence of F1's in my study, an observation shared by previous studies with limited post-F1 identification abilities (Ballinger et al. 1992; Derr 1991). It would also help explain the 39% of non-hybrid deer with hybrid indexes between zero and one. These individuals were not admixed enough to meet our

thresholds but possessed one or more heterospecific alleles. They may descend from hybrid lineages that have since restored their fitness through a series of backcrosses. By recovering their ability to survive in the wild, they can integrate into the population, facilitating the introgression of any residual heterospecific alleles. The low levels of introgression in these individuals would require genome-level data to be quantified (Gompert et al. 2017). Future studies examining the rate of fitness return would be necessary to definitively confirm this theory.

Direct observational research of hybrids is limited by their rarity in the wild. These types of studies would be necessary to answer questions about hybrid fitness directly. One theme might be to investigate hybrid behaviour in situations that prompt highly differentiated responses in pure species. For example, recent evidence has shown that, unlike WT and many other large herbivores, MD migratory behaviour is not plastic (Sawyer et al. 2019). WT may be obligate or conditional migrants (the condition being winter severity) (Feiberg et al. 2008) but MD appear to observe fixed migration behaviour: from year to year, MD residents will remain residents and MD migrants will remain migrants. Hybrid migration behaviour is not known. Similarly, the anti-predator strategies of hybrids have not been described (see chapter 1). Whether observational studies such as these are feasible, or even necessary given their current low prevalence, remains to be seen.

Given the structure of the hybrid zone, neither parental species seems to be significantly harmed by the current amount of hybridization. Hybrid fertility is low, eliminating the risk of genetic swamping via hybrid swarm, and the parental species show low affinity for heterospecifics during breeding season, reducing the risk of demographic swamping (Airst

and Lingle 2019). The system most resembles a tension zone: occasional hybridization occurs in sympatric populations but is checked by strong reproductive barriers (Hewitt 1988; Hu 2005). Because the selective pressures on hybrids are both intrinsic and extrinsic (i.e. postzygotic and prezygotic), they will most likely remain constant, barring any extreme changes to the ecosystem. Likewise, heterospecific mating is rare enough that its effect on the interspecific spread of CWD is likely negligible.

These observations taken together make forecasting the outlook of the hybrid zone in Alberta simple: there is no reason the current hybridization dynamic should drastically change in the foreseeable future. Many of the ecological factors that limit contemporary hybridization are intrinsic and unlikely to be altered in the coming generations. For example, habitat segregation—a product of the innate and unique anti-predator strategies of each species—curbs resource competition, which in turn limits overall interspecific interactions (Anthony and Smith 1977). By all accounts early-generation hybrids are maladapted in a number of respects, which, if all else fails, exposes them to predation to a greater degree than their pure species parents (Lingle 1993; Robinson et al. 2002). The potential for adaptive introgression exists (such as introgression of MD-specific cold-tolerance into WT populations) but the current volume of interspecific gene flow will slow this process to a crawl even by evolutionary standards (Abbott et al. 2013; Berry et al. 2019). Drawing from these conclusions, I expect other regions of sympatry to observe comparable hybridization rates when examined with the tools developed here, with the exception of regional hotspots. Hybridization hotspots may be generated by a variety of mechanisms, including human-mediated habitat disturbance (Todesco et al. 2016). Hornbeck and Mahoney (2000) cited this

as one possible explanation for their elevated observed hybridization rates in southwest Alberta. We can also see isolated populations of admixture in a comparable system in the UK, where Japanese sika deer (*Cervus nippon*) were introduced prior to forming hybrid swarms with native red deer (*C. elaphus*) (Ba et al. 2018; Senn et al. 2009; 2010; Smith et al. 2018). Accelerating habitat fragmentation and alteration, both locally and globally, could potentially affect the future of the MD x WT hybrid zone by displacing populations and increasing resource competition (McQuillan and Rice 2015; Taylor et al. 2014; 2015; Whittaker and Lindzey 2001).

To summarize, gene flow between *Odocoileus* spp. in Alberta is minimal and appears to be independent of geographical area. Although hybridization is an inherently variable process, even between multiple instances of the same parental species, I predict that studies similar to ours carried out in other regions will yield similar findings because of the endogenous nature of many of the reproductive barriers between mule and white-tailed deer (Gompert and Buerkle 2016; Nolte et al. 2009; Rieseberg 2006).

References

Abadie, P., Roussel, G., Dencausse, B., Bonnet, C., Bertocchi, E., Louvet, J., ... Garnier-Géré, P. (2012). Strength, diversity and plasticity of postmating reproductive barriers between two hybridizing oak species (quercus robur L. and quercus petraea (matt) liebl.). Journal of Evolutionary Biology, 25(1), 157-173. doi:10.1111/j.1420-9101.2011.02414.x

Abbott, R., Albach, D., Ansell, S., Arntzen, J. W., Baird, S. J. E., Bierne, N., ... Zinner, D. (2013). Hybridization and speciation. Journal of Evolutionary Biology, 26(2), 229-246. doi:10.1111/j.1420-9101.2012.02599.x

Airst, J., & Lingle, S. (2019). Courtship strategies of white-tailed deer and mule deer males when living in sympatry. Behaviour, 156(3-4), 307-330. doi: 10.1163/1568539X-00003543

Allendorf, F. W., Leary, R. F., Spruell, P., & Wenburg, J. K. (2001). The problems with hybrids: Setting conservation guidelines doi:10.1016/S0169-5347(01)02290-X

Anderson, E. C., and Thompson, E. A. (2002). A model-based method for identifying species hybrids using multilocus genetic data. Genetics, 160(3), 1217. http://www.genetics.org/content/160/3/1217.abstract

Andersson, L. (1990). The driving force: Species concepts and ecology. Taxon, 39(3), 375-382. doi:10.2307/1223084

Anthony, R. G., & Smith, N. S. (1977). Ecological relationships between mule deer and white-tailed deer in southeastern arizona.Ecological Monographs, 47(3), 255-277. doi:10.2307/1942517

Aulchenko, Y. S., Ripke, S., Isaacs, A., and van Duijn, C. M. (2007). GenABEL: An R library for genome-wide association analysis. Bioinformatics, 23(10), 1294-1296. http://dx.doi.org/10.1093/bioinformatics/btm108

Ba, H., Li, Z., Yang, Y., & Li, C. (2018). Development of diagnostic SNP markers to monitor hybridization between sika deer (cervus nippon) and wapiti (cervus elaphus). G3-Genes Genomes Genetics, 8(7), 2173-2179. doi:10.1534/g3.118.200417

Baker, R. J., & Bradley, R. D. (2006). Speciation in mammals and the genetic species concept. Journal of Mammalogy, 87(4), 643-662. doi:10.1644/06-MAMM-F-038R2.1

Balao, F., Casimiro-Soriguer, R., García-Castaño, J. L., Terrab, A., & Talavera, S. (2015). Big thistle eats the little thistle: Does unidirectional introgressive hybridization endanger the conservation of onopordum hinojense? New Phytologist, 206(1), 448-458. doi:10.1111/nph.13156

Ballinger, S., Blankenship, L., Bickham, J., & Carr, S. (1992). Allozyme and mitochondrial DNA analysis of a hybrid zone between white-tailed deer and mule deer (odocoileus) in west texas. Biochemical Genetics, 30, 1-11. doi:10.1007/BF00554423

Berry, S. L., Shipley, L. A., Long, R. A., & Loggers, C. (2019). Differences in dietary niche and foraging behavior of sympatric mule and white-tailed deer. Ecosphere, 10(7), e02815. doi:10.1002/ecs2.2815

Beugin, M., Gayet, T., Pontier, D., Devillard, S., & Jombart, T. (2018). A fast likelihood solution to the genetic clustering problem. Methods in Ecology and Evolution, 9(4), 1006-1016. doi:10.1111/2041-210X.12968

Bierne, N., David, P., Langlade, A., & Bonhomme, F. (2002). Can habitat specialisation maintain a mosaic hybrid zone in marine bivalves? Marine Ecology Progress Series, 245, 157-170. doi:10.3354/meps245157

Bierne, N., Gagnaire, P., & David, P. (2013). The geography of introgression in a patchy environment and the thorn in the side of ecological speciation. Current Zoology, 59(1), 72-86. doi:10.1093/czoolo/59.1.72

Bishop, M., Kappes, S., Keele, J., Stone, R., Sunden, S., Hawkins, G., … Beattie, C. W. (1994). A genetic-linkage map for cattle. Genetics, 136(2), 619-639. PubMed ID: 7908653

Bolger, A. M., Lohse, M., and Usadel, B. (2014). Trimmomatic: A flexible trimmer for illumina sequence data. Bioinformatics, 30(15), 2114-2120. doi: 10.1093/bioinformatics/btu170

Bouzat, J. L., Johnson, J. A., Toepfer, J. E., Simpson, S. A., Esker, T. L., & Westemeier, R. L. (2009). Beyond the beneficial effects of translocations as an effective tool for the genetic restoration of isolated populations. Conservation Genetics, 10(1), 191-201. doi:10.1007/s10592-008-9547-8

Bradley, R. D., Bryant, F. C., Bradley, L. C., Haynie, M. L., Baker, R. J., and Jones, C. A. (2003). Implications of hybridization between white-tailed deer and mule deer. The Southwestern Naturalist, 48(4), 654-660. doi: 10.1894/0038-4909(2003)048<0654:IOHBWD>2.0.CO;2

Brauning, R., Fisher, P., Mcculloch, A., J Smithies, ,Russell, Ward, J., Bixley, M., … McEwan, J. (2015). Utilization of high throughput genome sequencing technology for large scale

single nucleotide polymorphism discovery in red deer and canadian elk. bioRxiv 027318. https://doi.org/10.1101/027318.

Buerkle, C. A. (2005). Maximum-likelihood estimation of a hybrid index based on molecular markers. Molecular Ecology Notes, 5(3), 684-687. doi:10.1111/j.1471-8286.2005.01011.x

Buerkle, C. A., Wolf, D. E., & Rieseberg, L. H. (2003). The origin and extinction of species through hybridization. In C. A. Brigham, & M. W. Schwartz (Eds.), Population viability in plants: Conservation, management, and modeling of rare plants (pp. 117-141). Berlin, Heidelberg: Springer Berlin Heidelberg. doi:10.1007/978-3-662-09389-4_5

Burke, J. M., & Arnold, M. L. (2001). Genetics and the fitness of hybrids. Annual Review of Genetics, 35(1), 31-52.

Carr, S., and Hughes, G. (1993). Direction of introgressive hybridization between species of north-American deer (Odocoileus) as inferred from mitochondrial-cytochrome-B sequences. Journal of Mammalogy, 74(2), 331-342. doi: 10.2307/1382388

Carr, S. M., Ballinger, S. W., Derr, J. N., Blankenship, L. H., and Bickham, J. W. (1986). Mitochondrial DNA analysis of hybridization between sympatric white-tailed deer and mule deer in west texas. Proc Natl Acad Sci USA, 83(24), 9576. doi: 10.1073/pnas.83.24.9576

Casas, F., Mougeot, F., Sánchez-Barbudo, I., Dávila, J. A., & Viñuela, J. (2012). Fitness consequences of anthropogenic hybridization in wild red-legged partridge (alectoris rufa, phasianidae) populations. Biological Invasions, 14(2), 295-305. doi:10.1007/s10530-011-0062-3

Cathey, J., Bickham, J., and Patton, J. (1998). Introgressive hybridization and nonconcordant evolutionary history of maternal and paternal lineages in north American deer. Evolution, 52(4), 1224-1229. doi: 10.2307/2411253

Cingolani, P., Platts, A., Wang, L. L., Coon, M., Nguyen, T., Wang, L., … Ruden, D.M. (2012). A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of drosophila melanogaster strain w(1118); iso-2; iso-3. Fly, 6(2), 80-92. doi:10.4161/fly.19695

Colby, D. W., & Prusiner, S. B. (2011). Prions. Cold Spring Harbor Perspectives in Biology, 3(1), a006833. doi:10.1101/cshperspect.a006833

Coleman, R. R., Gaither, M. R., Kimokeo, B., Stanton, F. G., Bowen, B. W., & Toonen, R. J. (2014). Large-scale introduction of the indo-pacific damselfish abudefduf vaigiensis into hawai'i promotes genetic swamping of the endemic congener A. abdominalis. Molecular Ecology, 23(22), 5552-5565. doi:10.1111/mec.12952

Collinge, J. (2001). Prion diseases of humans and animals: Their causes and molecular basis. Annual Review of Neuroscience, 24(1), 519-550. doi:10.1146/annurev.neuro.24.1.519

Cronin, M. A. (1991). Mitochondrial and nuclear genetic relationships of deer (odocoileus spp.) in western north america. Canadian Journal of Zoology, 69(5), 1270-1279. doi:10.1139/z91-179

Cronin, M. A., Vyse, E. R., & Cameron, D. G. (1988). Genetic relationships between mule deer and white-tailed deer in montana. The Journal of Wildlife Management, 52(2), 320-328. doi:10.2307/3801243

Cullingham, C. I., Cooke, J. E., K., Dang, S., and Coltman, D. W. (2013). A species-diagnostic SNP panel for discriminating lodgepole pine, jack pine, and their interspecific hybrids. Tree Genetics and Genomes, 9(4), 1119-1127. doi: 10.1007/s11295-013-0608-x

Cullingham, C. I., Merrill, E. H., Pybus, M. J., Bollinger, T. K., Wilson, G. A., and Coltman, D. W. (2010). Broad and fine-scale genetic analysis of white-tailed deer populations: Estimating the relative risk of chronic wasting disease spread. Evolutionary Applications, 4(1), 116-131. doi: 10.1111/j.1752-4571.2010.00142.x

Cullingham, C. I., Nakada, S. M., Merrill, E. H., Bollinger, T. K., Pybus, M. J., and Coltman, D. W. (2011). Multiscale population genetic analysis of mule deer (Odocoileus hemionus hemionus) in western Canada sheds new light on the spread of chronic wasting disease. Canadian Journal of Zoology, 89(2), 134-147. doi: 10.1139/Z10-104

Daum, J. M., Davis, L. R., Bigler, L., and Woodhams, D. C. (2012). Hybrid advantage in skin peptide immune defenses of water frogs (Pelophylax esculentus) at risk from emerging pathogens. Infection Genetics and Evolution, 12(8), 1854-1864. doi: 10.1016/j.meegid.2012.07.024

DePristo, M. A., Banks, E., Poplin, R. E., Garimella, K. V., Maguire, J. R., Hartl, C., ... Daly, M. J. (2011). A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nature Genetics, 43(5), 491-498. doi:10.1038/ng.806

Derr, J. (1991). Genetic interactions between white-tailed and mule deer in the southwestern united states. The Journal of Wildlife Management, 55, 228. doi:10.2307/3809144

DeWoody, J., Honeycutt, R., and Skow, L. (1995). Microsatellite markers in white-tailed deer. Journal of Heredity, 86(4), 317-319. doi: 10.1093/oxfordjournals.jhered.a111593

Dobzhansky, T. (1936). Studies on hybrid sterility. II. localization of sterility factors in drosophila pseudoobscura hybrids. Genetics, 21(2), 113-135.

Dowling, T., and Secor, C. (1997). The role of hybridization and introgression in the diversification of animals. Annual Review of Ecology and Systematics, 28, 593-619. doi: 10.1146/annurev.ecolsys.28.1.593

Duenez-Guzman, E. A., Mavarez, J., Vose, M. D., and Gavrilets, S. (2009). Case studies and mathematical models of ecological speciation. 4. Hybrid speciation in butterflies in a jungle. Evolution, 63(10), 2611-2626. doi: 10.1111/j.1558-5646.2009.00756.x

Edmands, S., Northrup, S. L., and Hwang, A. S. (2009). Maladapted gene complexes within populations of the intertidal copepod Tigriopus californicus? Evolution, 63(8), 2184-2192. doi: 10.1111/j.1558-5646.2009.00689.x

Federal, Provincial, and Territorial Governments of Canada, 2014 2012 Canadian Nature Survey: Awareness, participation, and expenditures in nature-based recreation, conservation, and subsistence activities. Ottawa, ON: Canadian Councils of Resource Ministers.

Fernandez, M. E., Goszczynski, D. E., Liron, J. P., Villegas-Castagnasso, E. E., Carino, M. H., Ripoli, M. V., … Giovambattista, G. (2013). Comparison of the effectiveness of microsatellites and SNP panels for genetic identification, traceability and assessment of parentage in an inbred angus herd. Genetics and Molecular Biology, 36(2), 185-U194. doi: 10.1590/S1415-47572013000200008

Fieberg, J., Kuehn, D. W., & DelGiudice, G. D. (2008). Understanding variation in autumn migration of northern white-tailed deer by long-term study. Journal of Mammalogy, 89(6), 1529-1539. doi:10.1644/07-MAMM-A-277.1

Forrester, T. D., & Wittmer, H. U. (2013). A review of the population dynamics of mule deer and black-tailed deer odocoileus hemionus in north america. Mammal Review, 43(4), 292-308.

Frankham, R., Ballou, J.D., Eldridge, M.D.B., Lacy, R.C., Ralls, K., Dudash, M.R., & Fenster, C.B. (2011). Predicting the probability of outbreeding depression. Conservation Biology, 25(3), 465-475. doi:10.1111/j.1523-1739.2011.01662.x

Gabriel, S., Ziaugra, L., & Tabbaa, D. (2009). SNP genotyping using the sequenom MassARRAY iPLEX platform. *Current Protocols in Human Genetics, 60*(1), 2.12. 1-2.12. 18.

Gompert, Z., & Buerkle, C. A. (2010). INTROGRESS: A software package for mapping components of isolation in hybrids. Molecular Ecology Resources, 10(2), 378-384. doi:10.1111/j.1755-0998.2009.02733.x

Gompert, Z., & Buerkle, C. A. (2016). What, if anything, are hybrids: Enduring truths and challenges associated with population structure and gene flow. Evolutionary Applications, 9(7), 909-923. doi:10.1111/eva.12380

Gompert, Z., Mandeville, E. G., & Buerkle, C. A. (2017). Analysis of population genomic data from hybrid zones. Annual Review of Ecology, Evolution, and Systematics, 48(1), 207-229. doi:10.1146/annurev-ecolsys-110316-022652

Goudet, J. (2005). Hierfstat, a package for r to compute and test hierarchical F-statistics. Molecular Ecology Notes, 5(1), 184-186. doi:10.1111/j.1471-8286.2004.00828.x

Gourbière, S., and Mallet, J. (2010). Are species real? The shape of the species boundary with exponential failure, reinforcement, and the missing snowball. Evolution, 64(1), 1-24. doi: 10.1111/j.1558-5646.2009.00844.x

Grabenstein, K. C., & Taylor, S. A. (2018). Breaking barriers: Causes, consequences, and experimental utility of human-mediated hybridization doi: https://doi.org/10.1016/j.tree.2017.12.008

Habib, T. J., Merrill, E. H., Pybus, M. J., and Coltman, D. W. (2011). Modelling landscape effects on density-contact rate relationships of deer in eastern alberta: Implications for chronic wasting disease. Ecological Modelling, 222(15), 2722-2732. doi: 10.1016/j.ecolmodel.2011.05.007

Haldane, J. (1922). Sex ratio and unisexual sterility in hybrid animals. Journal of Genetics, 12(2), 101-109. doi: 10.1007/BF02983075

Haley, N. J., and Hoover, E. A. (2015). Chronic wasting disease of cervids: Current knowledge and future perspectives. Annual Review of Animal Biosciences, 3(3), 305-325. doi: 10.1146/annurev-animal-022114-111001

Hall, R. J., Hastings, A., and Ayres, D. R. (2006). Explaining the explosion: Modelling hybrid invasions. Proceedings of the Royal Society B-Biological Sciences, 273(1592), 1385-1389. doi: 10.1098/rspb.2006.3473

Harrison, R. G., & Larson, E. L. (2014). Hybridization, introgression, and the nature of species boundaries. Journal of Heredity, 105(S1), 795-809. doi:10.1093/jhered/esu033

Hassanin, A., Delsuc, F., Ropiquet, A., Hammer, C., van Vuuren, B. J., Matthee, C., Ruiz-Garcia, M., … Couloux, A. (2012). Pattern and timing of diversification of Cetartiodactyla (Mammalia, Laurasiatheria), as revealed by a comprehensive analysis of mitochondrial genomes. Comptes Rendus Biologies, 335(1), 32-50. doi: 10.1016/j.crvi.2011.11.002

Haynes, G. D., and Latch, E. K. (2012). Identification of novel single nucleotide polymorphisms (SNPs) in deer (Odocoileus spp.) using the BovineSNP50 BeadChip. Plos One, 7(5) doi: 10.1371/journal.pone.0036536

Hegarty, M. J., & Hiscock, S. J. (2008). Genomic clues to the evolutionary success of polyploid plants. Current Biology, 18(10), R435-R444. doi:10.1016/j.cub.2008.03.043

Hewitt, D. (Ed.). (2011). Biology and management of white-tailed deer (1st ed.). Boca Raton, FL: CRC Press.

Hewitt, G. M. (1988). Hybrid zones- Natural laboratories for evolutionary studies. Trends in Ecology and Evolution, 3(7), 158-167. doi: https://doi.org/10.1016/0169-5347(88)90033-X

Hewitt, G. M. (2001). Speciation, hybrid zones and phylogeography — or seeing genes in space and time. Molecular Ecology, 10(3), 537-549. doi:10.1046/j.1365-294x.2001.01202.x

Hornbeck, G. E., and Mahoney, J. M. (2000). Introgressive hybridization of mule deer and white-tailed deer in southwestern alberta. Wildlife Society Bulletin, 28(4), 1012-1015. doi: 10.2307/3783860

Hudson, R. R., & Coyne, J. A. (2002). Mathematical consequences of the genealogical species concept. Evolution, 56(8), 1557-1565. doi:10.1111/j.0014-3820.2002.tb01467.x

Hughes, G. A., & Carr, S. M. (1993). Reciprocal hybridization between white-tailed deer (odocoileus virginianus) and mule deer (O. hemionus) in western canada: Evidence from serum albumin and mtDNA sequences. Canadian Journal of Zoology, 71(3), 524-530. doi:10.1139/z93-073

Hu, X. (2005). Tension versus ecological zones in a two-locus system. Theoretical Population Biology, 68(2), 119-131. doi:10.1016/j.tpb.2005.02.003

Jeffery, N. W., DiBacco, C., Wringe, B. F., Stanley, R. R. E., Hamilton, L. C., Ravindran, P. N., Bradbury, I. R. (2017). Genomic evidence of hybridization between two independent invasions of european green crab (Carcinus maenas) in the northwest atlantic. Heredity, 119(3), 154-165. doi: 10.1038/hdy.2017.22

Johnson, S. E., Delmore, K. E., Brown, K. A., Wyman, T. M., and Louis, E. E., Jr. (2016). Niche divergence in a brown lemur (Eulemur spp.) hybrid zone: Using ecological niche models to test models of stability. International Journal of Primatology, 37(1), 69-88. doi: 10.1007/s10764-015-9872-y

Jombart, T. (2008). Adegenet: A R package for the multivariate analysis of genetic markers. Bioinformatics, 24(11), 1403-1405. doi:10.1093/bioinformatics/btn129

Jombart, T., & Ahmed, I. (2011). Adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. Bioinformatics, 27(21), 3070-3071. doi:10.1093/bioinformatics/btr521

Jones, K., Levine, K., and Banks, J. (2000). DNA-based genetic markers in black-tailed and mule deer for forensic applications. California Fish and Game, 86(2), 115-126. https://www.cabdirect.org/cabdirect/abstract/20013155304

Jun, G., Wing, M. K., Abecasis, G. R., and Kang, H. M. (2015). An efficient and scalable analysis framework for variant extraction and refinement from population-scale DNA sequence data. Genome Research, 25(6), 918-925. doi:10.1101/gr.176552.114

Kim, M., Cui, M., Cubas, P., Gillies, A., Lee, K., Chapman, M. A., . . . Coen, E. (2008). Regulatory genes control a key morphological and ecological trait transferred between species. Science, 322(5904), 1116-1119. doi:10.1126/science.1164371

Kirkpatrick, M., & Ravigné, V. (2002). Speciation by natural and sexual selection: Models and experiments. The American Naturalist, 159, S22-S35. doi:10.1086/338370

Kunkel, K. E., & Mech, L. D. (1994). Wolf and bear predation on white-tailed deer fawns in northeastern minnesota. Canadian Journal of Zoology, 72(9), 1557-1565.

Lamer, J. T., Ruebush, B. C., Arbieva, Z. H., McClelland, M. A., Epifanio, J. M., and Sass, G. G. (2015). Diagnostic SNPs reveal widespread introgressive hybridization between introduced bighead and silver carp in the mississippi river basin. Molecular Ecology, 24(15), 3931-3943. doi: 10.1111/mec.13285

Lamer, J. T., Sass, G. G., Boone, J. Q., Arbieva, Z. H., Green, S. J., and Epifanio, J. M. (2014). Restriction site-associated DNA sequencing generates high-quality single nucleotide polymorphisms for assessing hybridization between bighead and silver carp in the united states and china. Molecular Ecology Resources, 14(1), 79-86. doi: 10.1111/1755-0998.12152

Latch, E. K., Heffelfinger, J. R., Fike, J. A., and Olin, R. J. (2009). Species-wide phylogeography of north American mule deer (Odocoileus hemionus): Cryptic glacial refugia and postglacial recolonization. Molecular Ecology, 18(8), 1730-1745. doi: 10.1111/j.1365-294X.2009.04153.x

Latch, E. K., Kierepka, E. M., Heffelfinger, J. R., & Rhodes, O. E. (2011). Hybrid swarm between divergent lineages of mule deer (odocoileus hemionus). Molecular Ecology, 20(24), 5265-5279. doi:10.1111/j.1365-294X.2011.05349.x

Leary, R. E., Gould, W. R., & Sage, G. K. (1996). Success of basibranchial teeth in indicating pure populations of rainbow trout and failure to indicate pure populations of westslope cutthroat trout. North American Journal of Fisheries Management, 16(1), 210-213. doi:10.1577/1548-8675(1996)016<0210:SOBTII>2.3.CO;2

Levin, D. A., Francisco-Ortega, J., & Jansen, R. K. (1996). Hybridization and the extinction of rare plant species. Conservation Biology, 10(1), 10-16. Retrieved from http://www.jstor.org/stable/2386938

Li H. (2013) Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. Available from: http://arxiv.org/abs/1303.3997

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Martin, G. … Durbin, R. (2009). The sequence Alignment/Map format and SAMtools. Bioinformatics, 25(16), 2078-2079. doi: 10.1093/bioinformatics/btp352

Lingle, S. (1993). Escape gaits of white-tailed deer, mule deer, and their hybrids: Body configuration, biomechanics, and function. Canadian Journal of Zoology, 71(4), 708-724. doi: 10.1139/z93-095

Lingle, S. (2001). Anti-predator strategies and grouping patterns in white-tailed deer and mule deer. *Ethology, 107*(4), 295-314. doi:10.1046/j.1439-0310.2001.00664.x

Lingle, S. (2002). Coyote predation and habitat segregation of white-tailed deer and mule deer. Ecology, 83(7), 2037-2048. doi:10.1890/0012-9658(2002)083[2037:CPAHSO]2.0.CO;2

Lingle, S., Rendall, D., and Pellis, S. M. (2007). Altruism and recognition in the antipredator defence of deer: 1. Species and individual variation in fawn distress calls. Animal Behavior. 73(5), 897-905. doi: 10.1016/j.anbehav.2006.11.003

Lingle, S., Rendall, D., Wilson, W. F., DeYoung, R. W., and Pellis, S. M. (2007). Altruism and recognition in the antipredator defence of deer: 2. Why mule deer help nonoffspring fawns. Animal Behavior. 73(5), 907-916. doi: 10.1016/j.anbehav.2006.11.004

Lohr, J. N., and Haag, C. R. (2015). Genetic load, inbreeding depression, and hybrid vigor covary with population size: An empirical evaluation of theoretical predictions. Evolution, 69(12), 3109-3122. doi: 10.1111/evo.12802

Ma, J., & Wang, F. (2014). Prion disease and the "protein-only hypothesis". Essays Biochem, 56, 181. Retrieved from http://essays.biochemistry.org/content/56/181.abstract

Mallet, J. (2005). Hybridization as an invasion of the genome. Trends in Ecology and Evolution, 20(5), 229-237. doi: 10.1016/j.tree.2005.02.010

Martin, M. (2011). CUTADAPT removes adapter sequences from high-throughput sequencing reads. EMBnet.Journal, 17(10-2) doi: 10.14806/ej.17.1.200

Martin, M. D., & Mendelson, T. C. (2018). Hybrid sterility increases with genetic distance in snubnose darters (percidae: Etheostoma). Environmental Biology of Fishes, 101(2), 215-221. doi:10.1007/s10641-017-0672-4

Martinsen, G., Whitham, T., Turek, R., & Keim, P. (2001). Hybrid populations selectively filter gene introgression between species. Evolution, 55(7), 1325-1335. doi:10.1111/j.0014-3820.2001.tb00655.x

McClymont, R., Fenton, M., & Thompson, J. (1982). Identification of cervid tissues and hybridization by serum-albumin. Journal of Wildlife Management, 46(2), 540-544. doi:10.2307/3808677

McDevitt, A. D., Mariani, S., Hebblewhite, M., Decesare, N. J., Morgantini, L., Seip, D., . . . Musiani, M. (2009). Survival in the rockies of an endangered hybrid swarm from diverged caribou (rangifer tarandus) lineages. Molecular Ecology, 18(4), 665-679. doi:10.1111/j.1365-294X.2008.04050.x

McQuillan, M. A., & Rice, A. M. (2015). Differential effects of climate and species interactions on range limits at a hybrid zone: Potential direct and indirect impacts of climate change. Ecology and Evolution, 5(21), 5120-5137. doi:10.1002/ece3.1774

M'Gonigle, L. K., & FitzJohn, R. G. (2010). Assortative mating and spatial structure in hybrid zones. Evolution, 64(2), 444-455. doi:10.1111/j.1558-5646.2009.00838.x

Miller, M., Williams, E., McCarty, C., Spraker, T., Kreeger, T., Larsen, C., & Thorne, E. (2000). Epizootiology of chronic wasting disease in free-ranging cervids in colorado and wyoming.Journal of Wildlife Diseases, 36(4), 676-690. doi:10.7589/0090-3558-36.4.676

Miller, M. W., & Williams, E. S. (2003). Horizontal prion transmission in mule deer. Nature, 425(6953), 35-36. doi:10.1038/425035a

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

Miller, M. W., Wolfe, L. L., Sirochman, T. M., Sirochman, M. A., Jewell, J. E., and Williams, E. S. (2012). Survival patterns in white-tailed and mule deer after oral inoculation with a standardized, conspecific prion dose. Journal of Wildlife Diseases, 48(2), 526-529. doi: 10.7589/0090-3558-48.2.526

Mooney, H. A., & Cleland, E. E. (2001). The evolutionary impact of invasive species. Proceedings of the National Academy of Sciences, 98(10), 5446-5451. doi:10.1073/pnas.091093398

Muirhead, C. A., & Presgraves, D. C. (2016). Hybrid incompatibilities, local adaptation, and the genomic distribution of natural introgression between species. The American Naturalist, 187(2), 249-261. doi:10.1086/684583

Muller, H. (1942). Isolating mechanisms, evolution, and temperature. Biol.Symp., 6, 71-125. Retrieved from https://ci.nii.ac.jp/naid/10024791088/en/

Nalls, A. V., McNulty, E., Powers, J., Seelig, D. M., Hoover, C., Haley, N. J., . . . Mathiason, C. K. (2013). Mother to offspring transmission of chronic wasting disease in reeves's muntjac deer. Plos One, 8(8), e71844. doi: 10.1371/journal.pone.0071844

Ng, P. C., and Kirkness, E. F. (2010). Whole genome sequencing. Genetic variation: Methods and protocols, 628, 215-226. doi: 10.1007/978-1-60327-367-1_12

Nielsen, E. E., Bach, L. A., and Kotlicki, P. (2006). HYBRIDLAB (version 1.0): A program for generating simulated hybrids from population samples. Molecular Ecology Notes, 6(4), 971-973. doi: 10.1111/j.1471-8286.2006.01433.x

Nolte, A., Gompert, Z., & Buerkle, C. (2009). Variable patterns of introgression in two sculpin hybrid zones suggest that genomic isolation differs among populations. Molecular Ecology, 18(12), 2615-2627. doi:10.1111/j.1365-294X.2009.04208.x

Nosil, P. (2008). Speciation with gene flow could be common. Molecular Ecology, 17(9), 2103-2106. doi:10.1111/j.1365-294X.2008.03715.x

O'BRIEN, S. J., & MAYR, E. (1991). Bureaucratic mischief: Recognizing endangered species and subspecies. Science, 251(4998), 1187-1188. doi:10.1126/science.251.4998.1187

Oliveira, R., Randi, E., Mattucci, F., Kurushima, J., Lyons, L. A., & Alves, P. (2015). Toward a genome-wide approach for detecting hybrids: Informative SNPs to detect introgression between domestic cats and european wildcats (felis silvestris). Heredity, 115(3), 195-205. doi: 10.1038/hdy.2015.25

Orr, H. A., & Presgraves, D. C. (2000). Speciation by postzygotic isolation: Forces, genes and molecules. Bioessays, 22(12), 1085-1094. doi:10.1002/1521-1878(200012)22:12<1085::AID-BIES6>3.0.CO;2-G

Orr, H., & Turelli, M. (2001). The evolution of postzygotic isolation: Accumulating dobzhansky-muller incompatibilities. Evolution, 55(6), 1085-1094. doi:10.1111/j.0014-3820.2001.tb00628.x

Patterson, B. D., Ceballos, G., Sechrest, W., Tognelli, M. F., Brooks, T., Luna, L., Ortega, P., Salazar, I., Young, B. E. (2003) Digital distribution maps of the mammals of the western hemisphere, version 1.0. NatureServe, Arlington, Virginia, USA.

Petigara, M., Dridi, C., and Unterschultz, J. (2011). The economic impacts of chronic wasting disease and bovine spongiform encephalopathy in Alberta and the rest of Canada. Journal of Toxicology and Environmental Health-Part A-Current Issues, 74(22-24), 1609-1620. doi: 10.1080/15287394.2011.618987

Powell, J. H., Amish, S. J., Haynes, G. D., Luikart, G., and Latch, E. K. (2016). Candidate adaptive genes associated with lineage divergence: Identifying SNPs via next-generation targeted resequencing in mule deer (Odocoileus hemionus). Molecular Ecology Resources, 16(5), 1165-1172. doi: 10.1111/1755-0998.12572

Price, T. and Bouvier, M. (2002). The evolution of F-1 postzygotic incompatibilities in birds. Evolution, 56(10), 2083-2089. doi:10.1111/j.0014-3820.2002.tb00133.x

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. Genetics, 155(2), 945-959.

Qvarnström, A., Haavie, J., Sæther, S.A., Eriksson, D., & Part, T. (2006). Song similarity predicts hybridization in flycatchers. Journal of Evolutionary Biology, 19(4), 1202-1209. doi:10.1111/j.1420-9101.2006.01140.x

Randi, E., Hulva, P., Fabbri, E., Galaverni, M., Galov, A., Kusak, J., . . . Caniglia, R. (2014). Multilocus detection of wolf x dog hybridization in italy, and guidelines for marker selection. Plos One, 9(1), e86409. doi:10.1371/journal.pone.0086409

Remington, C. L. (1968). Suture-zones of hybrid interaction between recently joined biotas. In T. Dobzhansky, M. K. Hecht & W. C. Steere (Eds.), Evolutionary biology: Volume 2 (pp. 321-428). Boston, MA: Springer US. doi:10.1007/978-1-4684-8094-8_8

Rhymer, J. M., and Simberloff, D. (1996). Extinction by hybridization and introgression. Annual Review of Ecology and Systematics, 27(1), 83-109. doi: 10.1146/annurev.ecolsys.27.1.83

Richards, B. (2018). Distribution of Chronic Wasting Disease in North America. U.S. Geological Survey. Accessed from https://www.usgs.gov/media/images/distribution-chronic-wasting-disease-north-america-0

Rieseberg, L. H. (2006). Hybrid speciation in wild sunflowers. Annals of the Missouri Botanical Garden, 93(1), 34-49. doi:10.3417/0026-6493(2006)93[34:HSIWS]2.0.CO;2

Roberts, D.G., Gray, C.A., West, R.J., & Ayre, D.J. (2010). Marine genetic swamping: Hybrids replace an obligately estuarine fish. Molecular Ecology, 19(3), 508-520. doi:10.1111/j.1365-294X.2009.04501.x

Robinson, H. S., Wielgus, R. B., & Gwilliam, J. C. (2002). Cougar predation and population growth of sympatric mule deer and white-tailed deer. Canadian Journal of Zoology, 80(3), 556-568. doi:10.1139/z02-025

Ross, C., & Harrison, R. (2002). A fine-scale spatial analysis of the mosaic hybrid zone between gryllus firmus and gryllus pennsylvanicus. Evolution, 56(11), 2296-2312. doi:10.1554/0014-3820(2002)056[2296:AFSSAO]2.0.CO;2

Rowe, S., Clarke, S., Van Stijn, T., Hyndman, D., Ward, J., McEwan, K., Dodds, k., … Asher, G. (2015). Developing genomic tools in the new zealand deer industry. New Zealand Society of Animal Production, 75, 91-93.

Rusek, J., Ayan, G. B., Turko, P., Tellenbach, C., Giessler, S., Spaak, P., and Wolinska, J. (2015). New possibilities arise for studies of hybridization: SNP-based markers for the multi-species Daphnia longispina complex derived from transcriptome data. Journal of Plankton Research, 37(3), 626-635. doi: 10.1093/plankt/fbv028

Russell, T., Cullingham, C., Kommadath, A., Stothard, P., Herbst, A., & Coltman, D. (2019). Development of a novel mule deer genomic assembly and species-diagnostic SNP panel for assessing introgression in mule deer, white-tailed deer, and their interspecific hybrids. G3: Genes|genomes|genetics, 9(3), 911-919. doi:10.1534/g3.118.200838

Russo, I. M., Hoban, S., Bloomer, P., Kotze, A., Segelbacher, G., Rushworth, I., . . . Bruford, M. W. (2019). "Intentional genetic manipulation' as a conservation threat. Conservation Genetics Resources, 11(2), 237-247. doi:10.1007/s12686-018-0983-6

Sawyer, H., Merkle, J. A., Middleton, A. D., Dwinnell, S. P. H., & Monteith, K. L. (2019). Migratory plasticity is not ubiquitous among large herbivores. Journal of Animal Ecology, 88(3), 450-460. doi:10.1111/1365-2656.12926

Schwenk, K., Brede, N., and Streit, B. (2008). Introduction. Extent, processes and evolutionary impact of interspecific hybridization in animals. Philosophical Transactions of the Royal Society B-Biological Sciences, 363(1505), 2805-2811. doi: 10.1098/rstb.2008.0055

Seehausen, O. (2004). Hybridization and adaptive radiation. Trends in Ecology and Evolution, 19(4), 198-207. doi: 10.1016/j.tree.2004.01.003

Senn, H. V., & Pemberton, J. M. (2009). Variable extent of hybridization between invasive sika (cervus nippon) and native red deer (C-elaphus) in a small geographical area. Molecular Ecology, 18(5), 862-876. doi:10.1111/j.1365-294X.2008.04051.x

Senn, H. V., Swanson, G. M., Goodman, S. J., Barton, N. H., & Pemberton, J. M. (2010). Phenotypic correlates of hybridisation between red and sika deer (genus cervus). Journal of Animal Ecology, 79(2), 414-425. doi:10.1111/j.1365-2656.2009.01633.x

Stelfox, B. J. and Adamczewski, J. (1993). Hoofed mammals of Alberta (2nd ed.) Edmonton, Alberta, Canada: Lone Pine Publishing.

Stephens, M., Clipperton, N., and Bernie, M. (2009). Subspecies-informative SNP assays for evaluating introgression between native golden trout and introduced rainbow trout. Molecular Ecology Resources, 9(1), 339-343. doi: 10.1111/j.1755-0998.2008.02407.x

Stubblefield, S. S., Warren, R. J., & Murphy, B. R. (1986). Hybridization of free-ranging white-tailed and mule deer in texas. The Journal of Wildlife Management, 50(4): 688-690. doi: 10.2307/3800982

Swenson, N., & Howard, D. (2005). Clustering of contact zones, hybrid zones, and phylogeographic breaks in north america. American Naturalist, 166(5), 581-591. doi:10.1086/491688

Tamgüney, G., Miller, M. W., Wolfe, L. L., Sirochman, T. M., Glidden, D. V., Palmer, C., . . . Prusiner, S. B. (2009). Asymptomatic deer excrete infectious prions in faeces. Nature, 461(7263), 529-532. doi:10.1038/nature08289

Taylor, S. A., Larson, E. L., & Harrison, R. G. (2015). Hybrid zones: Windows on climate change. Trends in Ecology & Evolution, 30(7), 398-406. doi:10.1016/j.tree.2015.04.010

Taylor, S. A., White, T. A., Hochachka, W. M., Ferretti, V., Curry, R. L., & Lovette, I. (2014). Climate-mediated movement of an avian hybrid zone. Current Biology, 24(6), 671-676. doi:10.1016/j.cub.2014.01.069

Todesco, M., Pascual, M. A., Owens, G. L., Ostevik, K. L., Moyers, B. T., Hubner, S., . . . Rieseberg, L. H. (2016). Hybridization and extinction. Evolutionary Applications, 9(7), 892-908. doi:10.1111/eva.12367

Tokarska, M., Marshall, T., Kowalczyk, R., Wójcik, J. M., Pertoldi, C., Kristensen, T. N., Loeschcke, V., … Bendizen, C. (2009). Effectiveness of microsatellite and SNP markers for parentage and identity analysis in species with low genetic diversity: The case of European bison. Heredity, 103, 326. doi: 10.1038/hdy.2009.73

Twyford, A. D. and Ennos, R. A. (2011). Next-generation hybridization and introgression. Heredity, 108, 179. doi: 10.1038/hdy.2011.68

U.S. Department of the Interior, U.S. Fish and Wildlife Service, and U.S. Department of Commerce, U.S. Census Bureau. 2016 National Survey of Fishing, Hunting, and Wildlife-Associated Recreation.

Vähä, J., and Primmer, C. (2006). Efficiency of model-based bayesian methods for detecting hybrid individuals under different hybridization scenarios and with different numbers of loci. Molecular Ecology, 15(1), 63-72. doi: 10.1111/j.1365-294X.2005.02773.x

Van Valen, L. (1976). Ecological species, multispecies, and oaks. Taxon, 25(2), 233-239. doi:10.2307/1219444

Vignal, A., Milan, D., SanCristobal, M., and Eggen, A. (2002). A review on SNP and other types of molecular markers and their use in animal genetics. Genetics, Selection, Evolution : GSE, 34(3), 275-305. doi: 10.1186/1297-9686-34-3-275

Wang, I. J., & Bradburd, G. S. (2014). Isolation by environment. Molecular Ecology, 23(23), 5649-5662. doi:10.1111/mec.12938

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. Evolution, 38(6), 1358-1370. doi:10.1111/j.1558-5646.1984.tb05657.x

Whittaker, D. G., & Lindzey, F. G. (2001). Population characteristics of sympatric mule and white-tailed deer on rocky mountain arsenal, colorado. The Journal of Wildlife Management, 65(4), 946-952. doi:10.2307/3803043

Wiley, C., Qvarnström, A., Andersson, G., Borge, T., and Saetre, G. (2009). Postzygotic isolation over multiple generations of hybrid descendants in a natural hybrid zone: How well do single-generation estimates reflect reproductive isolation? Evolution, 63(7), 1731-1739. doi: 10.1111/j.1558-5646.2009.00674.x

Williams, E. (2005). Chronic wasting disease. Veterinary Pathology, 42(5), 530-549. doi: 10.1354/vp.42-5-530

Williams, E. S., Miller, M. W., Kreeger, T. J., Kahn, R. H., and Thorne, E. T. (2002). Chronic wasting disease of deer and elk: A review with recommendations for management. The Journal of Wildlife Management, 66(3), 551-563. doi: 10.2307/3803123

Wilson, G. A., Nakada, S. M., Bollinger, T. K., Pybus, M. J., Merrill, E. H., and Coltman, D. W. (2009). Polymorphisms at the PRNP gene influence susceptibility to chronic wasting disease in two species of deer (Odocoileus spp.) in Western Canada. Journal of

Toxicology and Environmental Health-Part A-Current Issues, 72(17-18), 1025-1029. doi: 10.1080/15287390903084264

Wilson, G., Strobeck, C., Wu, L., and Coffin, J. (1997). Characterization of microsatellite loci in caribou Rangifer tarandus, and their use in other artiodactyls. Molecular Ecology, 6(7), 697-699. doi: 10.1046/j.1365-294X.1997.00237.x

Wishart, W. D. (1980). Hybrids of white-tailed and mule deer in Alberta. Journal of Mammalogy, 61(4), 716-719. doi: 10.2307/1380318

Wishart, W. D., Hrudka, F., Schmutz, S. M., and Flood, P. F. (1988). Observations on spermatogenesis, sperm phenotype, and fertility in white-tailed X mule deer hybrids and a yak X cow hybrid. Canadian Journal of Zoology, 66(7), 1664-1671. doi: 10.1139/z88-240

Wolf, D., Takebayashi, N., and Rieseberg, L. (2001). Predicting the risk of extinction through hybridization. Conservation Biology, 15(4), 1039-1053. doi: 10.1046/j.1523-1739.2001.0150041039.x

Wood, A. K., Hamlin, K. L., Mackie, R. J., & Montana, W. D. (1989). Ecology of sympatric populations of mule deer and white-tailed deer in a prairie environment. Wildlife Division, Montana Dept. of Fish, Wildlife & Parks. Retrieved from https://www.biodiversitylibrary.org/item/137531

Wringe, B. F., Stanley, R. R. E., Jeffery, N. W., Anderson, E. C., and Bradbury, I. R. (2017). hybriddetective: A workflow and package to facilitate the detection of hybridization using genomic data in r. Molecular Ecology Resources, 17(6), 275-284. doi: 10.1111/1755-0998.12704

Zhou, Y., Duvaux, L., Ren, G., Zhang, L., Savolainen, O., & Liu, J. (2016). Importance of incomplete lineage sorting and introgression in the origin of shared genetic variation between two closely related pines with overlapping distributions. Heredity, 118, 211. doi:10.1038/hdy.2016.72