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Association Mapping of Genetic Risk Factors for Chronic Wasting Disease in Wild Deer

by

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Dedication

To my parents, Yutaka and Yuko Matsumoto.

Abstract

Association Mapping of Genetic Risk Factors for Chronic Wasting Disease in Wild Deer

Chronic wasting disease (CWD) is a fatal transmissible spongiform encephalopathy affecting North American cervids. I assessed the feasibility of association mapping for CWD genetic risk factors in wild white-tailed deer and mule deer using a high density microsatellite map of cattle. I chose a panel of 215 bovine microsatellite markers from three homologous deer linkage groups predicted to contain candidate genes for CWD. These markers had a low cross-species amplification rate (28 %) and showed weak linkage disequilibrium (< 1 cM). Markers near the prion protein gene (*PRNP*) and the neurofibromin 1 gene (*NF1*) were significantly associated with CWD status. Association of CWD with *PRNP* has been previously documented; however, this is the first time an association between the *NF1* region and CWD has been reported. More accurate and powerful association mapping in these populations in the future will require much denser marker sets such as genome-wide single nucleotide polymorphism panels.

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List of Symbols and Abbreviations

bp ..... base pairs

BSE ..... bovine spongiform encephalopathy

Bta ..... bovine chromosome

CJD ..... Creutzfeldt-Jakob disease

cM ..... centimorgan

CNS ..... central nervous system

CWD ..... chronic wasting disease

F_{IS} ..... within subpopulation *F* coefficient

F_{ST} ..... among subpopulation *F* coefficient

GPI ..... glycosyl phosphatidyl inositol

H_E ..... expected heterozygosity under HWE

H_O ..... observed heterozygosity

HSP90AA1 ..... heat shock protein 90kDa α (cytosolic) class A member 1 gene

HWE ..... Hardy-Weinberg equilibrium

kb ..... kilo (a thousand) base pairs

IL1B ..... interleukin-1β gene

IL1RN ..... interleukin-1 receptor antagonist gene

IL-1RA ..... interleukin-1 receptor antagonist

IL-1RI ..... interleukin-1 receptor type I

IL-1β interleukin-1β

LD ..... linkage disequilibrium

LG ..... linkage group

MAF ..... minor allele frequency

Mb ..... mega (a million) base pairs

MYA ..... million years ago

Nf1 ..... neurofibromin 1

NF1 ..... neurofibromin 1 gene

PCR ..... polymerase chain reaction

PRNP ..... prion protein gene

PrP ..... prion protein

PrP^{C} ..... cellular form of the prion protein

PrP^{Sc} ..... misfolded form of the prion protein

QTL ..... quantitative trait loci

RCF ..... relative centrifugal force

RPSA ..... ribosomal protein SA gene coding laminin receptor 1

SNP ..... single nucleotide polymorphism

SPRN ..... shadow of prion protein gene

Ta ..... annealing temperature

TD ..... touchdown PCR protocol

TSE ..... transmissible spongiform encephalopathy

vCJD ..... variant Creutzfeldt-Jacob disease

CHAPTER 1 - Introduction

1.1 Chronic Wasting Disease

Chronic wasting disease (CWD) was first recognized in 1967 as a fatal epidemic in captive mule deer (*Odocoileus hemionus*) at a research facility in Colorado, where affected animals exhibited terminal symptoms including weight loss, excessive salivation, ataxia and behavioural alteration (WILLIAMS 2005; MATHIASON *et al.* 2009). A decade later in 1978, CWD was identified as a transmissible spongiform encephalopathy (TSE), or a prion disease, based on characteristic spongiform degeneration and accumulation of protease resistant prion proteins (PrP^{Sc}) in the central nervous system (CNS) (WILLIAMS and YOUNG 1980; SPRAKER *et al.* 2002). The TSE was first brought to public's attention in 1980s with the UK outbreak of bovine spongiform encephalopathy (BSE) in cattle (*Bos taurus*) (WELLS *et al.* 1987) followed by the emergence of variant Creutzfeldt-Jacob disease (vCJD) among consumers of BSE-infected beef (BRUCE *et al.* 1997; HILL *et al.* 1997), though it has been known for many decades in domestic sheep (*Ovis aries*) and goats (*Capra hircus*) as scrapie and in humans as classical CJD and kuru (reviewed in AGUZZI 2006). CWD is so far the only TSE known to affect free-ranging animals (WILLIAMS 2005).

Starting in early 1980's, a large number of CWD cases were detected in wild mule deer, elk (*Cervus elaphus nelsoni*) and white-tailed deer (*O. virginianus*) east of the Rocky Mountains in Colorado and Wyoming (SPRAKER *et al.* 1997; MILLER *et al.* 2000). Extensive surveillance later detected the widespread distribution of CWD in both farm and wild cervids, ranging across 12 additional US states and two Canadian provinces (SIGURDSON and AGUZZI 2007). The discontinuous distribution suggests human transport of infected captive animals to be the likely cause for the spread and subsequent spillover into wild populations (WILLIAMS *et al.* 2002). Recently, a wild moose (*Alces alces shirasi*) shot in the CWD affected area of Colorado was diagnosed with CWD, totaling the number of affected species to four (BAETEN *et al.* 2007). Outside North America, an outbreak was reported in South Korean farms where captive elk imported from Canada were kept (SOHN *et al.* 2002; KIM *et al.* 2005).

CWD is highly contagious due to efficient horizontal transmission (MILLER and WILLIAMS 2003). Prevalence within major free-ranging foci in Colorado and Wisconsin was reported to reach as high as 10-30 % locally (WILLIAMS 2005; GREAR *et al.* 2006), and in captivity it can reach over 90 % (WILLIAMS 2005). While the exact mechanisms still remain elusive, transmission is known to occur both directly and indirectly via contaminated environment. Prion infectivity has been confirmed in a wide range of biological materials, including blood, saliva, and excreta (MATHIASON *et al.* 2006; HALEY *et al.* 2009). Decomposed carcasses are also infectious (MILLER *et al.* 2004). PrP^{Sc} accumulation is present in not only the CNS and lymphoid tissues but also various other organs, skeletal muscles, and fat tissues (ANGERS *et al.* 2006; SIGURDSON 2008; RACE *et al.* 2009). Furthermore, soil and water bodies appear to serve as sources of CWD spread as well as potential long-term reservoirs of infectious materials (SAUNDERS *et al.* 2008; NICHOLS *et al.* 2009). Efficient transmission has already hampered disease control efforts so much that eradication in areas where CWD has established is considered impractical (WILLIAMS *et al.* 2002). With the lack of medical treatment and disinfecting options, reducing prevalence through continuous surveillance and culling currently appears to be the only tool to limit further spread (WILLIAMS *et al.* 2002).

Struggling CWD management underscores substantial, potentially long-lasting socio-economic impact. Annual state-wide economic losses in Colorado and Wisconsin were estimated to surpass tens of millions of dollars as cervid game industries and individual farms were impacted and government expenditure increased (BISHOP 2004; SEIDL and KOONTZ 2004). Local businesses are also expected to suffer from decline in demands for cervid products as well as wildlife hunting and viewing participation (BISHOP 2004; SEIDL and KOONTZ 2004). After the vCJD outbreak, there are still significant public concerns for consumption of prion-associated meat, although experimental and circumstantial evidence suggests the risk of CWD transmission to humans to be low (e.g. WILLIAMS *et al.* 2002; BELAY *et al.* 2004; KONG *et al.* 2005). Yet public health agencies recommend avoiding contact with CWD contaminated materials (WILLIAMS *et al.* 2002; KAHN *et al.* 2004).

The extent of ecological impact is even less certain. Cross-species transmission is a particular concern given the likely exposure of high-dose infectious materials to predators and scavengers which also include domestic animals (JENNELLE *et al.* 2009; KRUMM *et al.* 2010). Indirect CWD exposure can also occur in co-inhabiting species as

seen in moose (BAETEN *et al.* 2007). For non-cervids, however, strong species-barriers appear to exist, and no natural cases have ever been found even in the long-established CWD endemic areas (reviewed in SIGURDSON 2008). Caribou (*Rangifer tarandus*), another cervid species inhabiting northern Canada and Alaska, might be at potential risk of epidemic because unlike moose their highly gregarious behaviour would facilitate rapid transmission (SIGURDSON 2008). Aside from cross-species transmission, little is known about other ecological impact of CWD. Where prevalence is high, however, increased direct and indirect (e.g. harvest and predation) mortality by CWD may significantly alter deer population dynamics and potentially affect predator-prey dynamics (GROSS and MILLER 2001; MILLER *et al.* 2008). Furthermore, as the affected cervid species are prime large herbivores in North America, the effect of CWD on their populations may inflict cascading disturbance to local ecosystems (MILLER *et al.* 2008). In summary, CWD is an emerging infectious disease for which socio-economic and ecological implications are huge but tools for effective control are lacking. Thus, increasing our understanding of the pathogenesis and transmission mechanisms is an urgent priority.

1.2 Transmissible Spongiform Encephalopathy Pathogenesis

Today TSEs are primarily agreed to be caused and transmitted by proteins devoid of nucleic acid, termed proteinaceous infectious particles, or prions (PRUSINER 1982). The infectious prion is a misfolded conformer (PrP^{Sc}) of the cellular prion protein (PrP^{C}) and exhibits distinct biochemical properties including increased β-sheet content, reduced solubility, partial protease resistance, and formation of aggregates called amyloid (COHEN and PRUSINER 1998). Pathogenic PrP^{Sc} invade host organisms and propagate by converting host-expressed PrP^{C} into the aberrant conformation. While the molecular mechanism underlying this process is still undetermined, predominant views include autocatalytic refolding of PrP^{C} by PrP^{Sc} and progressive polymerization of PrP^{C} onto oligomeric PrP^{Sc} seeds (reviewed in AGUZZI *et al.* 2008b). Other neurodegenerative diseases caused by similar protein misfolding include Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis, all of which are considered to be non-transmissible and have genetic and/or sporadic origins (reviewed in ROSS and POIRIER 2004). In addition to the infectious origin of TSEs, there are sporadic forms such as sporadic CJD, thought to result from rare spontaneous misfolding of PrP^{C},

and genetic forms such as familial CJD, Gerstmann-Sträussler-Scheinker disease, and fatal familial insomnia, all of which result from various mutations in the prion protein gene (*PRNP*) leading to PrP^{C} misfolding (reviewed in AGUZZI *et al.* 2008a).

Significant effort has been put into understanding TSE pathogenesis, but the exact molecular mechanisms underlining many key events are still not well understood. Following oral ingestion, PrP^{Sc} invade hosts via the digestive tract probably through intestinal epithelial cells (HEPPNER *et al.* 2001). From there, they likely enter the nervous system through nerve ends, often following a stage of peripheral PrP^{Sc} accumulation in lymphoid tissues (reviewed in AGUZZI *et al.* 2008b). Yet the precise role of the immune system and molecular mechanisms of PrP^{Sc} transport remain unknown (AGUZZI *et al.* 2008b). Furthermore, the exact molecular link between the neurodegeneration and the accumulation of PrP^{Sc} in the CNS is still under intensive investigation (AGUZZI *et al.* 2008b). Some evidence appear to suggest PrP^{Sc} itself may not be neurototoxic (BRANDNER *et al.* 1996; MALLUCCI *et al.* 2003) but instead the loss or alteration of still undetermined PrP^{C} functions might be the direct or indirect cause of degeneration (reviewed in WESTERGARD *et al.* 2007; AGUZZI *et al.* 2008b). Finally, TSEs exhibit distinct 'strains'. They can be identified by stably propagated differences in incubation periods, clinical and pathological profiles, and biochemical properties of PrP^{Sc} (reviewed in MORALES *et al.* 2007). Strains are believed to be coded by conformational variation of PrP^{Sc}, but the molecular underpinnings of this phenomenon are still elusive (MORALES *et al.* 2007).

Identification of the physiological functions of PrP^{C} has been and still is one of the major goals in prion research as it may lead to understanding the neurodegenerative mechanisms (AGUZZI *et al.* 2008b). PrP^{C} is mainly found as a glycosyl phosphatidyl inositol (GPI) anchored membrane protein with an extracellular peptide chain and is localized in lipid rafts (STAHL *et al.* 1987). PrP^{C} expression is known to begin in early stages of embryo development, and in adults it is primarily expressed in the CNS (reviewed in WESTERGARD *et al.* 2007). The functions of PrP^{C}, however, have been enigmatic. For example, *PRNP* knockout mice have shown no salient phenotypic deficits (BUELER *et al.* 1992) whereas mammalian *PRNP* sequences are highly conserved, indicating an important physiological role (WOPFNER *et al.* 1999). As a protein localized in the extracellular membrane surface, roles in signaling and trafficking are suggested to be most plausible. Intensively studied areas include binding and uptake of copper ions

and signaling roles in neuroprotection against apoptotic and oxidative stress (reviewed in WESTERGARD *et al.* 2007; AGUZZI *et al.* 2008a). Other evidence suggests potential involvement in neuronal growth and survival, synaptic functions, and cell adhesion; however, the clear picture of any single function is yet to be defined (reviewed in WESTERGARD *et al.* 2007; AGUZZI *et al.* 2008a). A plethora of potential interacting partners of PrP^C have also been identified, but physiological pathways in which they may be involved have been difficult to pinpoint (reviewed in AGUZZI *et al.* 2008a). In summary, the precise functions of PrP^C as well as many key pieces of the pathogenesis are yet to be uncovered. Consequently, no effective prophylactic treatment or cure has been found for TSEs (AGUZZI *et al.* 2008b).

1.3 Objective – Searching for Genetic Risk Factors for CWD

While experimental studies on molecular mechanisms have played a major role in advancing prion research, another potentially powerful approach is to look for genetic risk factors from an epidemiological standpoint. Identification of novel risk factors may lead to elucidating key pathways in prion pathogenesis and potentially revealing new therapeutic targets. Moreover, understanding the genetic basis of susceptibility differences in naturally infected deer might also help provide molecular tools to inform CWD management (NAKADA 2009). A number of studies have investigated genetic risk factors for TSEs, and the usefulness of this approach is expected to improve dramatically in upcoming years as more genomic resources become available.

Genetic risk factors have been usually studied using quantitative trait loci (QTL) mapping and candidate gene association tests. *PRNP* is the most prominent candidate risk factor studied to date, and varying degrees of associations between *PRNP* genotypes and TSE outcomes have been identified in all affected species (e.g. O'ROURKE *et al.* 1999; GOLDMANN *et al.* 2005; JEWELL *et al.* 2005; SANDER *et al.* 2005; AGUZZI 2006; JOHNSON *et al.* 2006). However, the molecular mechanism underlying the pathological differences is still not clear, and the effects of *PRNP* genotypes appear to vary considerably depending on strains (reviewed in MORALES *et al.* 2007). Investigation of other candidate genes has also been limited because of the lack of general understanding of the pathogenesis and PrP^C physiology (e.g. MARCOS-CARCAVILLA *et al.* 2007; MARCOS-CARCAVILLA *et al.* 2008b; BLANCHONG *et al.* 2009). QTL studies, on the other hand, mapped a large number of candidate risk regions to mice, sheep and cattle

chromosomes, which suggests the presence of many other important genetic risk factors (e.g. STEPHENSON *et al.* 2000; LLOYD *et al.* 2001; ZHANG *et al.* 2004; MORENO *et al.* 2008). However, this method has not been fully effective in pinpointing risk genes due to inconsistent results from different prion strains and animal breeds (e.g. STEPHENSON *et al.* 2000; LLOYD *et al.* 2001; MANOLAKOU *et al.* 2001) and a lack of resolution since QTL regions may harbour hundreds of genes.

A promising method for identifying genetic risk factors is association mapping, also called whole-genome or genome-wide association studies. Association mapping utilizes dense genetic markers and linkage disequilibrium (LD) in natural populations to indirectly detect disease associated loci (KRUGLYAK 1999). Because population level LD generally extends much shorter than linkage in family trees, association mapping can achieve high-resolution mapping not possible by QTL mapping (HIRSCHHORN and DALY 2005). With advancing high-throughput genotyping technologies, genome-wide association studies using > 100,000 single nucleotide polymorphism (SNP) markers are now possible in humans and many domesticated or model species (KRUGLYAK 1999). This approach was recently applied to CJD, vCJD, kuru, and BSE epidemics (MEAD *et al.* 2009; MURDOCH *et al.* 2010). These studies successfully identified highly localized genomic regions associated with elevated disease risks, some of which overlapped with known QTL and candidate regions while others were mapped to previously unidentified regions. Thus, it is clear that CWD, the least studied of all TSEs, can also benefit from association mapping. There may also be unique risk factors influencing CWD susceptibility and transmission in the wild that are not present in the other TSEs. Thus, association mapping can offer a way to comprehensively search the genome for important risk factors using samples obtained from CWD-affected natural populations.

Genomic resources for wild organisms are, however, considerably behind humans and model organisms. Wildlife, including most cervids, lack physical maps and automated SNP typing systems. A potential solution is to use a comparative approach whereby the genomic resources for an evolutionary related organism are applied to a wild counterpart. Here, the efficiency of marker transfer is determined by the evolutionary distance between the source and the target wild species (PRIMMER *et al.* 1996). For cervids, cattle are the closest relative with substantial genomic resources. Previous studies demonstrated large-scale chromosome conservation between the two groups (GALLAGHER *et al.* 1994; SLATE *et al.* 2002) as well as cross-species utility of bovine

microsatellite markers in cervids (SLATE *et al.* 1998). Currently there is a high density genetic map for cattle containing > 3,800 microsatellite markers (IHARA *et al.* 2004), which are expected to also amplify in deer at a reasonable rate (SLATE *et al.* 1998).

Therefore, the aim of my thesis was to examine the feasibility of association mapping for CWD in wild deer via the comparative use of the bovine microsatellite genetic map. To achieve this, I developed a marker panel for three predicted deer linkage groups (LGs) that I selected based on predicted assignment of candidate gene and QTL regions. In the first phase of this study (**Chapter 2**) I amplified bovine microsatellites from the selected LGs in deer, and assessed the efficiency of cross-species amplification. I then genotyped two CWD-affected deer populations, white-tailed deer from Wisconsin and mule deer from Saskatchewan, using the developed marker panels and estimated the levels of LD. These results allowed me to estimate the maximum number of bovine microsatellites that could be transferred to deer and the spacing of the markers required for uninterrupted coverage of the LGs. I also examined the level of population substructure in the target deer populations since it could confound the association results (PRITCHARD and DONNELLY 2001).

In the second phase (**Chapter 3**) I utilized these marker panels to test marker-CWD associations in case-control samples. At this point, it was clear that the bovine markers would not provide enough coverage: the first phase revealed a modest cross-species amplification rate and weak LD. However, each of my three candidate gene regions; *PRNP*, interleukin-1β and its receptor antagonist (*IL1B*/*IL1RN*), and neurofibromin 1 (*NF1*) regions, were tagged by markers located within the expected range of strong LD. Thus, I was able to indirectly interrogate these regions for CWD associations. Also, the ample of unlinked markers outside the candidate regions allowed me to assess the extent of confounding in my samples.

The main findings from my study will provide both a big picture and specific implications for the future prospects of genetic research in CWD. **Chapter 2** is a proof of principle test of association mapping in deer using currently available ungulate genomic resources, and also provides new data on the levels of LD in wild vertebrates. **Chapter 3** constitutes specific tests of CWD association at candidate genomic regions, and potentially leads to the identification of risk variants and their pathological significance.

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CHAPTER 2 - Cross-species Amplification of Bovine Microsatellite Markers and Estimation of Linkage Disequilibrium in Wild Deer

2.1 Introduction

Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE), or a prion disease, of North American cervids (family Cervidae), currently affecting captive and wild elk (*Cervus elaphus*), mule deer (*Odocoileus hemionus*), white-tailed deer (*O. virginianus*), and wild moose (*Alces alces*). TSEs are infectious, fatal neurodegenerative disorders also known in humans as Creutzfeldt-Jakob disease (CJD) and kuru, in sheep (*Ovis aries*) and goats (*Capra hircus*) as scrapie, and in cattle (*Bos taurus*) as bovine spongiform encephalopathy (BSE). The TSE's unconventional infectious agents were found to be a misfolded conformer (PrP^{Sc}) of the cellular prion proteins (PrP^{C}) (PRUSINER 1989). While accumulation of PrP^{Sc} in the central nervous system (CNS) is a key pathological feature, many underlying mechanisms of TSE pathogenesis including the normal physiological functions of PrP^{C} still remain elusive (reviewed in WESTERGARD *et al.* 2007; AGUZZI *et al.* 2008a). So far no effective means of prevention or treatment have been developed despite the decades of extensive research (AGUZZI and POLYMENIDOU 2004).

CWD is unique from other TSEs in its occurrence in the wild. Since its discovery, disease management has been severely impeded by efficient horizontal transmission of disease agents via environmental sources, and this has resulted in substantial economic losses to farming, gaming, and tourism industries (BISHOP 2004; SEIDL and KOONTZ 2004). Horizontal transmission is known to result from prion infectivity found in various tissues of infected animals and their remains which enter the environment (MILLER and WILLIAMS 2003). Infectious prions are also shed in blood and saliva during the subclinical stage (MATHIASON *et al.* 2006; MATHIASON *et al.* 2009) that often lasts over a few year period in naturally exposed animals (WILLIAMS 2005). Once in the environment, prions retain infectivity in soil for a prolonged period, aggravating the risk of exposure (MILLER *et al.* 2004; GEORGSSON *et al.* 2006). This also raises a great ecological concern for potential cross-species transmission into to co-inhabiting predators, scavengers, and grazers (JENNELLE *et al.* 2009). Futhermore, the variant CJD (vCJD) outbreaks in humans via the consumption of BSE-infected beef posed persisting

public health concerns for the undetermined risk of human exposure to CWD through consumption of venison, though evidence so far suggest it to be low (BELAY *et al.* 2004; KONG *et al.* 2005). These serious ramifications clearly make developing effective CWD mitigation measures an urgent priority, and to achieve this, filling in the gaps in the current understanding of the CWD pathogenesis is essential.

TSEs are known to be under the influence of genetic risk factors; identification of which could provide keys to revealing important elements of the pathogenesis. The most prominent factors identified to date are the effects of prion protein gene (*PRNP*) genotypes on relative host susceptibility. While some genotypes in humans (reviewed in AGUZZI 2006) and sheep (reviewed in HUNTER 2007) confer strong resistance to TSEs, *PRNP* polymorphisms in deer were found to be only weakly associated with disease outcomes, and susceptible alleles generally predominated in wild populations (O'ROURKE *et al.* 1999; JOHNSON *et al.* 2003; O'ROURKE *et al.* 2004; JEWELL *et al.* 2005; HAMIR *et al.* 2006; JOHNSON *et al.* 2006; PERUCCHINI *et al.* 2008; WILSON *et al.* 2009). Furthermore, the fact that *PRNP* only explained a fraction of the total genetic variance in TSEs (DIAZ *et al.* 2005; LLOYD and COLLINGE 2005) and that many other QTL and candidate genes have been discovered (e.g. STEPHENSON *et al.* 2000; LLOYD *et al.* 2001; MORENO *et al.* 2003; ZHANG *et al.* 2004; MEAD *et al.* 2009) suggest TSEs are complex diseases with many underlying genetic risk factors. CWD, however, is the least studied of all TSEs. No QTL mapping studies have been conducted, and the only other candidate gene studied so far, complement component C1q, was found not to be associated with the susceptibility in the wild (BLANCHONG *et al.* 2009). The exceptional transmissibility of CWD, however, suggests factors not present in other TSEs may also play a role. Therefore, the need for more comprehensive investigation of CWD risk factors is clear.

One powerful approach for tackling the genetic basis of a complex disease is association mapping, also known as genome-wide (i.e. whole genome) association studies. The principle population genetic concept underlying this approach is linkage disequilibrium (LD). It is broadly defined as a non-random association of alleles at two or more loci (LEWONTIN and KOJIMA 1960). In association mapping we are interested in LD caused by physical proximity of the loci in a genome. LD enables the detection of disease-associated loci by looking for allele frequency differences at nearby genetic markers between case and control groups. Thus, theoretically one can screen a dense set of markers across the whole genome with the goal of finding multiple, if not all, risk

genes associated with the disease (KRUGLYAK 1999). Association mapping in wild populations also presents several other advantages over linkage mapping and candidate gene studies. First, it circumvents experimental infections involving long incubation periods (WILLIAMS 2005) and associated ethical concerns. Second, it allows us to use samples of unrelated individuals from a naturally infected population, unlike linkage mapping that requires pedigree-based populations. Third, unlike candidate gene studies it requires no prior knowledge about biochemical pathways or gene functions possibly involved in the pathogenesis, and finally it allows high resolution mapping over large genomic regions (HIRSCHHORN and DALY 2005). For these reasons, I explored the feasibility of association mapping for identifying novel CWD risk factors in wild deer populations.

There are a few critical requirements for association mapping to function properly: 1) characterization of genetic structure in the target population; 2) high density genetic markers of known chromosomal positions; and 3) knowledge of the level of LD in the target population. As in any case-control association tests, the presence of population structure could seriously confound the association results: therefore it is necessary to delineate the potential structure in order to avoid spurious associations (PRITCHARD and DONNELLY 2001). Characterization of the level of LD in the target population is an essential prerequisite for determining appropriate marker density necessary for genome coverage. Since the population level LD is much shorter than linkage in family trees, association mapping requires substantially denser marker coverage than traditional linkage mapping (KRUGLYAK 1999). In recent years, this requirement has been fulfilled for humans and many livestock and model species as their genome sequences and haplotype maps of single nucleotide polymorphisms (SNPs) became available (e.g. ALTSHULER *et al.* 2005; LINDBLAD-TOH *et al.* 2005; GIBBS *et al.* 2009). This resulted in the emergence of genome-wide association studies for many complex diseases, including human TSEs and BSE (MEAD *et al.* 2009; MURDOCH *et al.* 2010). However, cervid genome sequences and SNP maps are still unavailable at present. In order to meet the requirement of high density genetic markers, I investigate the utility of a comparative approach, whereby genomic resources of a related model species are transferred to a target non-model species.

The most comprehensive cervid genomic resource available today is the genetic map of the subfamily Cervinae established by SLATE *et al.*(2002). It is derived from

interspecies hybrids between red deer (*c. elaphus*) and Père David's deer (*Elaphurus davidianus*) and contains > 600 markers of various types mapped at a mean interval of ~5.8 cM across 33 autosomal and sex linkage groups (LGs). The same study also established the chromosomal homology with cattle, sheep, and humans which provides a useful comparative framework. While the map's utility for association mapping is limited by the low density of microsatellite markers (153 markers across all LGs), the comparative approach can offer an effective and time-saving alternative to *de novo* isolation of microsatellite through more extensive cross-species amplification. Because the efficiency is determined by the genetic distance between a source and a target species (PRIMMER *et al.* 1996), I chose cattle (order Artiodacyla; family Bovidae) as the closest relative of Cervidae with the most abundant genomic resources. Previous studies illuminated the usefulness of bovine microsatellite markers in cervids with over 50 % polymorphic amplifications (ENGEL *et al.* 1996; KÜHN *et al.* 1996; SLATE *et al.* 1998). With a transfer rate as high as this, the genetic map of cattle containing ~3,800 microsatellite markers (IHARA *et al.* 2004) would have the potential to provide deer with a genetic map of a few centimorgan (cM) resolution.

The marker density required for association mapping fundamentally depends on the extent of LD in the target population. LD has been well characterized in humans and domestic animal populations using: formerly, microsatellite markers; and more recently, high density SNPs. In humans, LD is known to extend from a few to ~100 kb, and a panel of ~300,000–500,000 SNPs is typically required for a genome-wide association study (REICH *et al.* 2001; ARDLIE *et al.* 2002; WEISS and CLARK 2002; ALTSHULER *et al.* 2005). As demographic history affects the levels of LD observed in current populations, many domestic populations with small historical effective population sizes tend to have higher levels of LD compared to humans, not infrequently extending beyond 1 cM, and thus, requiring fewer markers for association mapping (SUTTER *et al.* 2004; LINDBLAD-TOH *et al.* 2005; AMARAL *et al.* 2008). High density SNPs also uncovered extensive haplotype block structures at fine-scales (<100 kb) which has great implications for careful marker selection (ALTSHULER *et al.* 2005; LINDBLAD-TOH *et al.* 2005; QANBARI *et al.* 2010). In wild vertebrates, however, LD studies are still limited in number and are largely restricted to low resolution microsatellites or SNPs in small genomic regions (MCRAE *et al.* 2002; BACKSTRÖM *et al.* 2006; SLATE and PEMBERTON 2007; LI and MERILA 2010). Exceptions to this were species for which SNP maps are available from

closely related domestic counterparts, namely wild murids (LAURIE *et al.* 2007), canids (GRAY *et al.* 2009), and sheep (MILLER *et al.* 2011). In cervids, LD has only been estimated from an island population of red deer, which revealed extensive LD over tens of centimorgans using microsatellites (SLATE and PEMBERTON 2007). Therefore, characterization of LD in CWD-affected deer populations provides novel insights to the levels of LD found in wild vertebrate populations, particularly those that are large-sized, genetically diverse, and distributed across relatively continuous landscapes (e.g. VAN DEN BUSSCHE *et al.* 2002; LATCH *et al.* 2009; NAKADA 2009). I expect these attributes of typical deer populations in North America to likely result in lower levels of LD compared to the younger, more isolated red deer populations investigated by SLATE and PEMBERTON (2007).

In this study, I aim to ascertain the feasibility of association mapping of novel CWD risk factors in wild deer using the comparative approach. To achieve this goal, I used CWD-affected white-tailed deer from Wisconsin and mule deer from Saskatchewan and evaluated: 1) the efficiency of the cross-species amplification of bovine microsatellite markers; 2) the presence of population structure; and 3) the levels of LD in the target populations. I expect the findings of this study to also illuminate the comparative genetics of artiodactyls and the levels of LD in wild vertebrate populations, and provide prospects for association mapping in wild, non-model species lacking substantial genomic resources.

2.2 Materials and Methods

2.2.1 Linkage Group Selection

I selected three red deer linkage groups (LGs) to investigate in this study based on two criteria: 1) predicted assignment of candidate genes and quantitative trait loci (QTL) previously identified for other TSEs; 2) high degree of conservation with cattle homologs. For the first criterion I identified, using established genome homology, bovine chromosomes that were suspected to harbor regions associated with BSE and scrapie. This included reports from QTL studies in mice, sheep, and cattle, as well as candidate gene association studies in humans, sheep, and cattle (**Table 2-1**). For the second criterion, homology between red deer LGs and cattle chromosomes (denoted as Bta) established by SLATE *et al.*(2002) was used to select red deer LGs with no or only

unambiguous rearrangements and sufficient microsatellite anchor markers (i.e. markers mapped in both red deer and cattle), which were used to confirm marker order conservation between the maps. Based on these criteria, I selected three red deer LGs and corresponding four cattle homologs: LG 23 (Bta 13), LG 11 (Bta 11) and LG 5 (Bta 17 and 19, Robertsonian-fused) (**Table 2-1**).

In order to use the red deer genetic map as the blueprint for white-tailed and mule deer, I confirmed gross homology of the selected LGs between the two cervid lineages. Earlier cytogenetic studies identified karyotypes of congeneric white-tailed and mule deer to be equivalent to each other ($2n = 70$), but they differed from that of red deer ($2n = 68$; FONTANA and RUBINI 1990; GALLAGHER *et al.* 1994). Although no direct cytogenetic comparisons were made with red deer, banding patterns between mule deer and cattle chromosomes have been compared previously (GALLAGHER *et al.* 1994), allowing me to make indirect comparisons. Based on GALLAGHER *et al.* (1994) the four cattle chromosomes I selected correspond to four separate chromosomes in mule deer, indicating the fusion of Bta17 and 19 observed in red deer is absent. Other than this aspect, evidence of the large-scale chromosome conservation between cattle and red deer (SLATE *et al.* 2002) and between cattle and mule deer (GALLAGHER *et al.* 1994) suggest that organizations of the three red deer LGs should be highly conserved in white-tailed and mule deer.

2.2.2 Study Area and Sample Selection

This study targeted wild deer populations from CWD-affected areas in Wisconsin and Saskatchewan. CWD has established widespread distribution in both areas since first detection nearly a decade ago (JOLY *et al.* 2003; KAHN *et al.* 2004). I obtained CWD negative white-tailed deer samples from Wisconsin: first, to screen a large number of bovine microsatellite markers for amplification; and second, to estimate the extent of LD in the population. Mule deer samples from Saskatchewan were subsequently used to screen cross-amplification of white-tailed deer-transferred bovine markers and then to estimate the extent of LD in the population.

2.2.2.1 White-tailed Deer Samples

White-tailed deer tissue samples were provided by the Wisconsin Department of Natural Resources through Dr. Michael Samuel, the collaborator at the University of Wisconsin – Madison. The population sample consisted of CWD negative deer (N = 184) collected across the CWD management zone during the 2002 hunting surveillance season (see Figure 1 in GREAR *et al.* 2006), which was initiated after the detection of the first wild CWD cases (JOLY *et al.* 2003). Each removed deer was registered with a sampling barcode number and information about the individual's age, sex, and kill location (GREAR *et al.* 2006). Kill locations were recorded on the basis of the Public Land Survey System (PLSS) of Wisconsin, which divides the state into 36 square-mile grids called townships, which are subdivided into 36 one-square mile sections (HANSON *et al.* 1996). The Wisconsin Veterinary Diagnostic Laboratory conducted the CWD testing on retropharyngeal lymph nodes and brain stem (obex) tissue by immunohistochemsitry or enzyme-linked immunosorbent assay (ELISA) (GREAR *et al.* 2006).

2.2.2.2 Mule Deer Samples

The Saskatchewan mule deer samples (N = 192) were selected from a pool of tissue samples that had been preserved from a previous landscape genetic study conducted by NAKADA (2009) at the University of Alberta. It was a subset of hunter submitted samples and retropharyngeal lymph node biopsies collected by the University of Saskatchewan and the Canadian Cooperative Wildlife Health Centre during the provincial disease control effort from 2001 to 2007 (NAKADA 2009). Each sample was accompanied by information about its age, sex, CWD status and sampling locations recorded in the Universal Transverse Mercator coordinates. A majority of samples were collected from the southern CWD range along the South Saskatchewan River Valley, whereas a minority belonged to the northern CWD range along the North Saskatchewan River (see Figure 1 in WILSON *et al.* 2009). CWD testing was performed by standard immunohistochemistry techniques using tonsil or retropharyngeal lymph node tissues (WILSON *et al.* 2009). I selected the sample set by matching CWD case-control pairs as it was designed to be subsequently analyzed for disease association in the second part of this thesis. Details of the matched case-control design are described in **Chapter 3**.

2.2.3 DNA Extraction

2.2.3.1 Phenol-chloroform Extraction – White-tailed Deer

DNA was extracted from the white-tailed deer tissue samples via the phenol-chloroform method at Dr. Samuel's laboratory in the Department of Wildlife Ecology at the University of Wisconsin–Madison. The samples were skeletal muscles stored frozen at -20°C, sealed and double-bagged in barcode-labeled plastic bags. As safety procedures, all organic extraction was conducted under a fume hood.

The tissue samples were prepared and digested as follows: 1) approximately 0.1 g of the frozen tissue was cut out on a glass plate, while sterilizing the cutting tools with 70 % ethanol after each sample; 2) tissues were placed in a 1.5 mL centrifuge tubes containing 600 µL of extraction buffer [20 mM Tris-chloride, pH 8.0; 20 mM EDTA, pH 8.0; 20 µl/mL RNase A, DNase-free (Fermentas UAB, Vilnius, Lithuania); 0.1 % SDS] and were incubated for 20 minutes at 65–67 °C in a water bath or an isotemp oven; 3) 20 µL of 20 mg/mL Proteinase K (Ambion, Inc., Austin, TX, USA) were added, mixed by inversion, and incubated overnight at 50–52 °C in an isotemp oven; 4) additional 20 µL of Proteinase K were added and incubated for one to two hours at 50–52 °C to ensure thorough digestion.

DNA was separated from the tissue digest via three rounds of organic extraction: 1) buffer saturated phenol was added to the lysed tissue until the tube was filled, gently mixed by inversion, and centrifuged at 7,000 RCF for 10 minutes, and this resulted in the separation of DNA into the aqueous top phase, other unwanted cellular materials into the bottom phase, and proteins into the interphase; 2) the aqueous phase was transferred into a new tube, and an equal volume of 1:1 mixture of phenol and chloroform was added, mixed by inversion, centrifuged at 7,000 RCF for five minutes to further remove the organic contaminants; 3) an equal volume of chloroform was added to transferred aqueous phase and centrifuged at 7,000 RCF for five minutes to ensure the removal of phenol residue.

Lastly DNA was precipitated and washed in ethanol as follows: 1)1/10 volume of 3 M sodium acetate and 2.25 volumes of 95 % ethanol were added to the resulting aqueous phase, mixed by inversion and centrifuged at full speed (13,000 RCF) for five minutes to obtain salt-bound DNA pellets; 2) after removing the supernatant with a vacuum aspirator or pipettors, 500 µL of 70 % ethanol was added to wash off the excess salt, and tubes were centrifuged at full speed for five minutes; 3) the supernatant was

removed, and the pellets in the tubes were left to dry in the isotemp oven for approximately 20 minutes. The extracted DNA was transported in the dried form to our lab at the University of Alberta, where it was eluted with 200 µL of miliQ H_2O.

2.2.3.2 Phenol-chloroform Extraction – Mule Deer

The Saskatchewan mule deer samples were provided as ethanol-fixed tissues stored at -20 °C. DNA extraction was conducted in our laboratory at the Department of Biological Sciences in the University of Alberta. The same phenol-chloroform protocol was performed with following changes to the reagents: RNase was excluded from the extraction buffer; 3 M sodium acetate was substituted with 3 M ammonium acetate; Proteinase K provided in QIAGEN DNeasy® Blood & Tissue Kit was used (QIAGEN, Hilden, Germany).

2.2.3.3 QIAGEN Kit Extraction – Cattle

DNA from a single cow was extracted to serve as a positive control in the amplification screening. A small amount of skeletal muscle tissue of a single cow was obtained from a local grocery store. DNA extraction was performed using QIAGEN DNeasy® Blood & Tissue Kit, following the instruction provided by the manufacturer. Final elution was made with miliQ H_2O instead of Buffer AE provided in the kit, in order to maintain consistency with the storage medium of the phenol-chloroform-extracted deer DNA.

2.2.3.4 DNA Quantification

I measured the concentrations of the miliQ H_2O-eluted DNA samples via spectrophotometry using Nanodrop^{TM} 2000 (Thermo Fisher Scientific Inc., Wilmington, DE, USA), following the manufacturer's instruction. DNA concentration was calculated in the instrument using the standard value of 50 µg/mL DNA = 1.0 A_{260}. Purity estimates were obtained using the ratio of A_{260} / A_{280}, where a ratio between 1.8 and 1.9 indicated high-purity DNA, and higher (>1.9) and lower (<1.5) ratios indicated potential contaminations by RNA and phenol/protein, respectively. I standardized the concentrations of DNA samples at ~20 ng/µL and stored them in sealed 96-well storage plates at -20 °C.

2.2.4 Bovine Microsatellite Screening in White-tailed Deer

2.2.4.1 Marker Selection and M13-Primer Labeling

I sampled microsatellite markers at an approximate interval of 2.5 cM from the high density bovine microsatellite map (IHARA *et al.* 2004) for the four selected cattle chromosomes. Additional markers were sampled from each of the predicted candidate regions (**Table 2-1**) to increase the marker density in these targeted regions. This resulted in a total set of 215 microsatellite markers with known bovine positions (53 markers for red deer LG23, 66 markers for LG11, and 96 markers for LG5).

M13 florescent primer labeling system (SCHUELKE 2000) was adopted for cost-effective screening of the large number of primer sets. This system allows incorporation of florescent labeled universal M13 primers into any unlabelled, marker-specific primer sets by adding a 18 base-pair (bp) M13 sequence (TGT AAA ACG ACG GCC AGT) to the 5' end of one of the primers. I obtained bovine primer sequences from IHARA *et al.* (2004) and purchased M13-modified primers (forward primers were modified) from Integrated DNA Technologies, Inc. (Coralville, IA, USA). M13 universal primers labelled with four florescent dyes (FAM™, VIC®, NED™, and PET®) were purchased from Applied Biosystems (Foster City, CA, USA) to be used with LIZ® labelled size standard in a G-5 dye set specified by the supplier.

2.2.4.2 Polymerase Chain Reaction

The bovine microsatellites primers were screened for polymerase chain reaction (PCR) amplification using a panel of six to seven white-tailed deer along with positive (cattle) and negative (miliQ H_2O) controls. White-tailed deer samples with the highest DNA yields were used in the panel. Screening PCR was attempted in a total volume of 15 µL, consisting of: 2.5 µL of 20 ng/µL template DNA (approximately 50 ng), 1.5 µL of 10× PCR buffer [100 mM Tris-Cl , pH 8.8; 500 mM KCl; 1 % Triton X-100™ (Sigma Aldrich, Saint Louis, MO, USA); 1.6 mg/mL BSA, nuclease free (Roche Diagnostic Corporation, Indianapolis, IN, USA)], 1.14 µL of 25 mM $MgCl_2$, 1.5 µL of 2mM each dNTP's (Fermentas UAB, Vilnius, Lithuania), 0.24 µL of marker specific primers [2.5 µM M13-modified forward primer, and 10 µM reverse primer], 0.24 µL of 10 µM dye labelled M13 primer, 0.1 µL of 5 U/µL Taq polymerase (prepared at the Department of

Biological Sciences, the University of Alberta), and 7.78 µL of miliQH_2O. Reactions were prepared on ice and were set up in 96 well reaction plates or 8-strip PCR tubes. Thermocycles were performed on Mastercycler® ep gradient (Eppendorf, Hamburg, Germany). The thermocyclers were pre-warmed to 94 °C denaturation temperature before the loading of the reaction plates to avoid nonspecific binding of the primers. The initial round of screening involved one standard cycling condition for all markers [one minute initial denaturation at 94 °C, 3 cycles of 30 second denaturation at 94 °C; 20 second annealing at 52 °C; 5 second extension at 72°C, 30 cycles of 15 second denaturation at 94 °C; 20 second annealing at 52 °C; 5 second extension at 72°C, and 15-minute final extension at 72 °C].

2.2.4.3 Fragment Analysis

A set of four PCR products labeled with each of FAM™, VIC®, NED™, and PET® (Applied Biosystems) were co-loaded for capillary electrophoresis on a 48-capillary 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA) serviced at the Molecular Biology Service Unit of the Department of Biological Sciences. PCR products were first combined and diluted so that each marker was 1/9 of the original concentration. Two µL of this mixed dilution product was loaded onto 8 µL of Hi-Di™ Formamide (Applied Biosystems, Foster City, CA, USA) containing × 0.0235 (0.188 µL) of GeneScan™ 500 LIZ® Size Standard (Applied Biosystems, Foster City, CA, USA) in a 96 well reaction plate, which resulted in a total of 1/45 dilution per marker. Prior to loading on the analyzer, the plates were spun to eliminate air bubbles and heated at 100 °C for two minutes to denature the amplified DNA. Plates were then run on the 48-capillary 3730 DNA analyzer by following the manufacturer's instruction.

Electropherograms were visualized and inspected for the amplification of microsatellite peaks using GeneMapper® Software v4.0 (Applied Biosystems, Foster City, CA, USA). The cattle positive control served as a reference for approximate allele size ranges and patterns of stuttering when successfully amplified, and when it failed to amplify, it indicated the inadequacy of current PCR conditions. The negative control (no template DNA) aided in identifying noises from true amplicons. Criteria for successful polymorphic amplification included evidence of variability (i.e. at least two alleles), heterozygotes, and unambiguous allele callings.

2.2.4.4 PCR Optimizations

When the initial screening resulted in nonspecific or no products, I optimized the PCR conditions by changing the annealing temperature (Ta) of the standard cycling condition described above (**Section 2.2.4.2**). Touchdown protocols (KORBIE and MATTICK 2008) were also employed to increase specificity and yields [Ta was dropped by 0.5 °C with each cycle for 15 cycles, and at the lowest reached Ta additional 15 cycles were performed]. The dilution factors for co-loading were adjusted to produce desirable peak intensity in resulting electropherograms. It should be noted the PCR optimization efforts varied across markers. More effort was put into markers exhibiting microsatellite-like peaks as well as those in the target regions, while little effort was put in if adjacent markers have already successfully amplified (see **Appendix I**).

2.2.5 Microsatellite Transfer from White-tailed Deer to Mule Deer

Based on the high degree of relatedness between the two deer species, I attempted an efficient development of the mule deer marker panel by screening only those markers that had been successfully genotyped in white-tailed deer. Seventy such markers were screened for amplification on a panel of seven mule deer and one white-tailed deer serving as a positive control. The first round of screening was done with each marker's optimal PCR condition used in white-tailed deer. Optimization effort was more rigorous than it was for white-tailed deer as the closer evolutionary distance meant higher degree of similarity in primer binding sequences, thus, higher cross-amplification success rate. The same PCR and fragment analysis methods as white-tailed deer were followed (see **Section 2.2.4**).

2.2.6 Genotyping

Both the white-tailed deer and the mule deer population samples were genotyped for markers that had amplified apparently variable microsatellite alleles in the screening panels. The optimal PCR conditions from the screening were followed (see **Table 2-2; Table 2-3)**. I co-loaded markers with non-overlapping size ranges to minimize bleed-over of intense florescent signals from one marker into the others. Genotyping of the electropherograms were conducted in Genemapper® Software v4.0, and all automated allele callings were visually inspected and corrected. I re-ran failed PCR in mule deer to

obtain genotypes for at least 97% of the samples. This sometimes involved re-optimization of the PCR conditions by lowering the Ta and increasing the Mg^{2+} concentration to 2.5 mM or 3.0 mM. I did not re-run failed PCR in white-tailed deer; however, most markers yielded genotypes for 90 to 98% of the samples in the initial population reactions. I compiled the genotype data in Microsoft® Excel and used the Excel Microsatellite Toolkit (PARK 2001) to check for errors in allele coding.

2.2.7 Diversity Statistics

I calculated basic population diversity statistics (number of alleles, observed and expected heterozygosity) for each locus in the Excel Microsatellite Toolkit. The observed heterozygosity (H_O) was calculated as the number of heterozygous individuals divided by the total number of individuals. The expected heterozygosity (H_E) was calculated using Nei's unbiased gene diversity (NEI 1978) defined as:

$$H_E = \frac{2N}{2N-1}\left(1 - \sum_{i=1}^{n} {p_i}^2\right) \qquad \text{Eq. 2-1}$$

where p_i^2 is the Hardy-Weinberg homozygote frequency of allele i, and N is the sample size.

F_{IS} is the within-population coefficient of the F-statistics that collectively describes genetic differentiation among multiple populations (WRIGHT 1951), and is also used to quantify heterozygote deficiency within a single population. Following NEI (1977)'s definition, F_{IS} of multiple populations can be expressed as a relationship between the average observed and the expected heterozygosities, shown as:

$$F_{IS} = \frac{H_S - H_O}{H_S} \qquad \text{Eq. 2-2}$$

where H_S and H_O are the weighted averages of within-population H_E and H_O, respectively. Thus, in the case of a single population, F_{IS} is simply calculated from H_E and H_O at each locus. Positive and negative F_{IS} respectively indicate deficiency and excess of heterozygotes within a population. I used Genepop v4.0 (ROUSSET 2008) to calculate F_{IS} per locus and averaged over loci for each species, using WEIR and COCKERHAM (1984)'s estimate. Finally I reported mean number of alleles, H_E, H_O, and F_{IS} over loci. From this calculation I excluded the outlying loci with suspected genotyping errors that are discussed next (see **Section 2.2.8**).

2.2.8 Detection of Allelic Dropout

Variation within primer binding sequences can cause allelic dropout and "noise" in the electropherogram (e.g. nonspecific amplification or ambiguous stuttering) and may introduce considerable errors in the genotype data. This problem is particularly prominent for cross-species amplification due to the higher degree of divergence between the primer and template DNA sequences. I used Hardy-Weinberg equilibrium (HWE) probability, tests of allele-specific heterozygote deficiency, and F_{IS} distribution to identify markers experiencing potential allelic dropout and excluded them from the subsequent analyses.

2.2.8.1 HWE Probability

I estimated HWE probability at each locus in Genepop v4.0. Calculations of the exact probabilities were performed through one of the two methods as described in the documentation of the software: the complete enumeration with less than five alleles, or a Markov chain (MC) algorithm with five or more alleles. Whenever the MC estimation was performed, I increased the batch number to 5,000 to assure an accurate estimation was reached. The significance level was adjusted by the Bonferroni correction to prevent inflation of Type I error in multiple testing.

2.2.8.2 Allele-Specific Heterozygote Deficiency

I used Micro-Checker (VAN OOSTERHOUT *et al.* 2004) to help identify genotyping errors caused by null alleles, stuttering, and large allele dropout. This program distinguishes the different sources of errors using patterns of allele-specific heterozygote deficiency under HWE assumption. More specifically, the program detects: 1) null alleles when heterozygote deficiency is evenly distributed across all observed alleles; 2) genotype scoring error due to stuttering when there is heterozygote deficiency between alleles differing by a single repeat unit and relative excess of large allele homozygotes; and 3) large allele dropout when homozygote excess is biased in shorter alleles (VAN OOSTERHOUT *et al.* 2004). Bonferroni correction was not used for this set of analyses due to the increased number of multiple testing, but the results were interpreted jointly with the other analyses to maintain correct Type I error rate.

2.2.8.3 F_{IS} Distribution

The HWE-based tests for detecting allelic dropout described above can be confounded if non-random mating exists within the populations. Therefore, I also used frequency distributions of F_{IS} (plotted in SigmaPlot v11.0; Systat Software Inc, San Jose, CA, USA) to delineate the baseline levels of deviation from the HWE within each population, and then identified markers with outlying F_{IS} using natural breaks in the distributions. These breaks roughly corresponded to one standard deviation from the mean.

2.2.9 Marker Panels and Density

For each species I produced a map of the three LGs with marker positions using MapChart v2.1 (VOORRIPS 2002). Positions were directly inferred from the bovine high density microsatellite map (IHARA *et al.* 2004). Mean marker interval for each LG and all LGs combined were also calculated.

2.2.10 Population Structure

Because cryptic population structure can result in spurious LD between unlinked markers and cause false association between markers and phenotypes in association studies (LANDER and SCHORK 1994; PRITCHARD and DONNELLY 2001), I tested the presence of population structure within the samples using a F_{IS} based permutation test and a Bayesian clustering method. For these analyses, I reduced the number of markers so that none of them were in significant LD ($p < 0.05$) with one another (see **Section 2.2.11.2** for the significance testing of LD). This resulted in datasets of 30 loci in white-tailed deer and 22 loci in mule deer.

2.2.10.1 Significance Testing of F_{IS}

I tested the significance of the deviation from HWE using a F_{IS} based permutation test implemented in Fstat v2.9.3 (GOUDET 1995). The test calculated F_{IS} values from permutated datasets, and the proportion of datasets with values as large as or larger than the F_{IS} of the original sample was output as the unbiased probability. Significance testing of F_{IS} over all loci was then calculated as the weighted average of the statistic obtained from each locus as outlined in the documentation of the program. I

conducted 600 and 440 permutations for the white-tailed deer and mule deer samples, respectively.

2.2.10.2 Bayesian Clustering Method

I used a Bayesian clustering method implemented in Structure v2.2.3 (PRITCHARD *et al.* 2000) to test for the signs of structure in my samples. For a pre-assigned number of subpopulations (*K*), this program simultaneously infers allele frequencies of each subpopulation and probabilities of individuals' ancestry to each subpopulation, by minimizing within-subpopulation LD and Hardy-Weinberg disequilibrium (HWD) via Malkov chain Monte Calro (MCMC) method (PRITCHARD *et al.* 2000). I employed an admixture and correlated allele frequency model from $K = 1$ to $K = 10$, each with five replicates using a run-length of 100,000 steps for a burn-in period and 100,000 steps for a parameter estimation period. As described by the program developers, mean posterior probabilities($\ln Pr(X|K)$), proportions of samples assigned to each subpopulation, and proportions of individuals' ancestry to each subpopulation were used as indications of the presence/absence of the population structures.

2.2.11 Linkage Disequilibrium

Common measures of LD such as *D'* (LEWONTIN 1964) and r^2 (HILL and ROBERTSON 1968) are useful for comparisons among studies but cannot be directly calculated from genotype data when haplotype phases are unknown. In this case, haplotype frequency or phase reconstructions can be done through maximum likelihood (EXCOFFIER and SLATKIN 1995) or Bayesian frameworks (STEPHENS *et al.* 2001). Such methods, however, are not always suitable for a small complex dataset with highly polymorphic loci because it may involve undesirable assumptions (e.g. random mating), high computational power and/or significant error rates (STEPHENS *et al.* 2001). As this was the case for my datasets, instead of estimating haplotypes I adopted genotype-based LD measures developed by WEIR (1979), called the composite measures.

2.2.11.1 Composite Correlation

I measured the levels of LD between syntenic markers using the square of the correlation coefficient (r^2) calculated from the composite measures (WEIR 1979). The

composite measures incorporate unresolved haplotype phases by using allelic associations *within genotypes* instead of *within haplotypes*. While the conventional haplotype-based LD is defined by $D_{AB} = p_{AB} - p_A p_B$ (LEWONTIN and KOJIMA 1960) with $p_{AB,}$ $p_{A,}$ and p_B representing frequencies of haplotype AB, allele A, and allele B, respectively, its composite analogue is defined by WEIR (1979) as:

$$\Delta_{AB} = p_{AB} + p_{A/B} - 2p_A p_B \qquad \text{Eq. 2-3}$$

Here, the alternative states of the alleles A and B within a genotype, namely AB (on the same haplotype) and A/B (on different haplotypes), are not distinguished but counted jointly. Under HWE, Δ_{AB} is equivalent to D_{AB} because $p_{A/B} - p_A p_B = 0$ (WEIR 1979). The correlation coefficient based on Δ is defined by WEIR (1979) as:

$$r^c{}_{AB} = \frac{\Delta_{AB}}{\sqrt{[p_A(1 - p_A) + D_A][p_B(1 - p_B) + D_B]}} \qquad \text{Eq. 2-4}$$

where D_A and D_B are single-locus HWD coefficients (i.e. $D_A = p_{AA} - {p_A}^2$), and the superscript *c* denotes the composite-based r as opposed to the haplotype based r. Again under HWE, $r^c{}_{AB}$ is equivalent to r_{AB} (WEIR 1979). I used multiallelic extension of $r^c{}_{AB}$ defined by ZAYKIN *et al.* (2008) as:

$$\bar{r}^2 = \frac{\sum\sum {r_{ij}^c}^2}{k\,m} \qquad \text{Eq. 2-5}$$

where $r^c{}_{ij}$ is the correlation coefficient of each pair of alleles (i, j) at the two loci, and k, m are numbers of alleles at the two loci. I performed the calculation of $\bar{r}^2$ in a program developed by ZAYKIN *et al.* (2008). Hereafter, $\bar{r}^2$ is simply referred to as composite r^2.

I plotted composite r^2 for each LG against predicted inter-marker distances directly taken from the bovine map using Sigma Plot v11.0. An exponential decay regression line defined as $y = y_0 + a\, e^{-bx}$ was fitted to each plot. This model described roughly linear initial decline of r^2 from its maximum value at 0 cM estimated by $y_0 + a$, which then slowly stabilizes to a minimum or background value of r^2 estimated by y_0. To describe the speed of decline over distance, I used a distance, denoted as $x_{1/2}$, at which r^2 declined to the half value between the initial maximum r^2 and the background minimum r^2, and was calculated from the regression line as $x_{1/2} = ln\left(\frac{1}{2}\right)/(-b)$.

2.2.11.2 Significance Testing of LD

The significance of composite LD between all syntenic and nonsyntenic marker pairs was tested in Genepop v4.0. The test was of the independence of genotypes in a two-locus contingency table via a log likelihood ratio statistic (*G*-test) using the MC algorithm (RAYMOND and ROUSSET 1995). In order to ensure appropriate sampling of the table space by the MC algorithm, I used 10,000 dememorization, 5,000 batches, and 5,000 iterations per batch, and confirmed that resulting standard error was sufficiently low (<<0.001) and number of switches sufficiently high (>>10,000) as suggested in the documentation of the program (RAYMOND and ROUSSET 1995).

I plotted negative log transformed *P*-values as a function of the predicted genetic distance in Sigma Plot v11.0. The predicted distances were inferred from the bovine map (IHARA *et al.* 2004). Background level of LD was established as a proportion of significant LD ($p < 0.05$) between nonsyntenic pairs, and I tested whether it significantly differed from the proportion expected by chance (i.e. Type I error rate of 0.05) via χ^2 test. I also summarized the proportions of significant ($p < 0.05$) syntenic marker pairs by predicted distance intervals and compared them against the background level using *Z*-test of two proportions in Sigma Plot v11.0. Bonferroni correction was used to account for multiple testing.

2.2.11.3 Pooling Rare Alleles

As high levels of genetic diversity were observed in both species, the significance testing of LD could suffer a loss of power and introduction of statistical errors via unobserved genotypes as well as extremely low expected frequencies. In order to assess the effect of having rare alleles on significance testing, I created a second dataset for each species where all rare alleles (frequency <0.05) at each locus were pooled whenever possible. When the summed frequency of rare alleles was still less than 0.05, I combined it with the allele having the next lowest frequency. I repeated the same LD testing in Genepop v4.0 (**Section 2.2.11.2**) and tested the mean difference in probabilities before and after the pooling using Wilcoxon Signed Rank Test, the nonparametric alternative to paired two sample *t*-test, in Sigma Plot v11.0.

2.3 Results

2.3.1 Cross-species Amplification

2.3.1.1 Bovine Microsatellite Screening in White-tailed Deer

The cross-species amplification rate of bovine markers in the white-tailed deer screening panel was 39.1 %, where 84 markers out of the total 215 amplified and were polymorphic. Of the remaining markers, 9.8 % amplified but appeared invariable, and 50.1 % amplified no product or only non-specific products within the range of PCR conditions tested. One marker showed evidence of high frequency null allele(s) in the screening panel. It should be noted that not all failed markers were followed up by the optimization (see **Appendix I**), thus, my estimate of the amplification rate could be conservative by a small degree.

2.3.1.2 Microsatellite Transfer from White-tailed Deer to Mule Deer

Of the 70 markers that had been successfully genotyped in the white-tailed deer population sample, 36 markers (51.4 %) amplified and were polymorphic in the mule deer screening sample under the same PCR conditions. Following optimization, an additional 17 polymorphic markers (24.3 %) amplified, bringing the success rate to 75.7 %. There were also a considerable number of markers (18.6 %) that amplified but were monomorphic, and only four markers (5.7 %) failed completely in the screening even after the significant optimization effort. Contrary to the screening in white-tailed deer, optimization in mule deer was intensive since we expected high cross-amplification success; thus, the estimated rate is expected to be more accurate.

2.3.2 Diversity Statistics

Population genotypes were obtained from 70 and 45 markers in white-tailed and mule deer, respectively, and their diversity statistics and loci means are shown in **Table 2-2** and **Table 2-3**. Despite having less than 200 samples per population, both species exhibited a high degree of genetic diversity, with the mean (± standard deviation) number of alleles per locus of 10.7 (± 6.3) and 7.8 (± 3.5), respectively (all means stated here exclude the outlying markers discussed in **Section 2.3.3**). The mean H_O was 0.70 (± 0.25) in white-tailed deer and 0.64 (± 0.19) in mule deer. The mean H_E was slightly higher than the observed in both species: 0.72 (± 0.23) in white-tailed deer and 0.65 (± 0.19) in mule

deer. The mean F_{IS} were both small but positive: 0.029 (± 0.042) in white-tailed deer and 0.019 (± 0.049) in mule deer.

2.3.3 Detection of Allelic Dropout

Markers experiencing potential allelic dropout and other genotyping complications were detected using the HWE tests, the tests of allele-specific heterozygote deficiency, and the F_{IS} distribution. Markers with significant deviation from HWE after Bonferroni correction are indicated in **Table 2-2** and **Table 2-3**, along with the specific sources of genotyping errors detected by Micro-Checker. Due to the significant positive mean F_{IS} found in both samples (see **Section 2.3.5**), I used natural breaks in the observed F_{IS} distribution, which approximately corresponded to one standard deviation from the mean, to draw a cutoff line for calling markers with serious genotyping issues (**Figure 2-1**). Based on this I excluded ten loci (F_{IS} > 0.15) in white-tailed deer and three loci (F_{IS} > 0.16) in mule deer (**Table 2-2; Table 2-3**). A majority of the F_{IS} outliers were also found significant for the HW deviation and the allele-specific heterozygote deficiency suggesting either null alleles or genotype calling errors due to stuttering.

2.3.4 Marker Density

The cross-amplification success rate from cattle to white-tailed deer was 32.6 % (70 markers) after the population genotyping and was reduced to 27.9 % (60 markers) with the exclusion of the F_{IS} outliers (**Table 2-2**). The transfer rate from white-tailed deer to mule deer was 64.3 % (45 markers) after the population genotyping and was reduced to 60.0 % (42 markers) with the exclusion of the F_{IS} outliers (**Table 2-3**).

The final marker panel for white-tailed deer was 60 markers with a mean (± standard deviation) predicted interval of 6.26 (± 5.28) cM (**Figure 2-2**; **Table 2-4**), and for mule deer it was 42 markers at 9.75 (± 8.11) cM (**Figure 2-3**; **Table 2-4**). There was variation in the mean predicted intervals among the three LGs, with LG23 having the smallest interval and LG5 having the largest interval in both species (**Table 2-4**). Variations in individual intervals were also large, ranging from 0 to 24.68 cM in white-tailed deer (**Figure 2-2**) and 0 to 29.49 cM in mule deer (**Figure 2-3**). There were total of 57 intervals in white-tailed deer, of which four were less than 1 cM and 32 were less than

5 cM. In mule deer there were 39 total intervals, and three were less than 1 cM and 15 were less than 5 cM.

2.3.5 Population Structure

The presence of population structure was assessed using F_{IS} and the Bayesian clustering method implemented in Structure v2.2.3. First, the small positive mean F_{IS} in both samples (**Table 2-2**; **Table 2-3**) was statistically significant ($p = 0.002$ in white-tailed deer and $p = 0.01$ in mule deer), indicating small degrees of non-random mating within each population. Second, using Structure v2.2.3. I detected no distinct subpopulations as the posterior probabilities (ln $Pr(X|K)$) were the highest for $K = 1$ in both species (**Figure 2-4**). Although $K = 2$ in white-tailed deer (**Figure 2-4a**) and $K = 2$ and 9 in mule deer (**Figure 2-4b**) resulted in similar levels of the posterior probability as to $K = 1$, they did not signify true clusters because: 1) nearly equal proportions ($1/K$) of the individuals were assigned to each inferred cluster; and 2) inferred ancestry of the individuals were also shared equally among the clusters (data not shown). Both of these features likely indicated the absence of real population structure (PRITCHARD *et al.* 2000).

2.3.6 Linkage Disequilibrium

2.3.6.1 Patterns of Composite r^2 by LGs

The levels of LD between pairs of syntenic markers were measured by composite r^2 (**Figure 2-5**). Both species exhibited overall low levels of r^2 (< 0.15) across all LGs, indicating weak correlation of genotypes between pairs of markers. However, non-random patterns of r^2 with the predicted marker distance were still observed in most LGs, where r^2 was highest near 0 cM and declined to a background level with distance.

To describe this trend I fitted an exponential decay regression line to individual LGs. All LGs except for LG5 of mule deer fitted the model relatively well with all parameters statistically significant ($p \ll 0.05$): however, due to high variability in the data the regression explained only a small fraction of the variation in r^2 ($R^2 < 0.2$). An exception to this was LG11 in mule deer, where nearly half the variation in r^2 could be explained (R^2=0.5; **Figure 2-5d**). I was unable to fit the regression to LG5 of mule deer due to the lack of data points at small distances (**Table 2-4**; **Figure 2-5f**).

The patterns of declining r^2 estimated from the fitted lines varied among LGs, although the background levels of r^2 were highly similar across all LGs, ranging from 0.072 to 0.076. The differences were seen in: 1) the inferred maximum r^2 at 0 cM; and 2) the rate of decline described by $x_{1/2}$. In white-tailed deer, LG5 appeared to have higher maximum r^2 at 0 cM (≈ 0.12) with a faster rate of decline ($x_{1/2}$ = 3.0 cM; **Figure 2-5e**) than both LG23 and 11, which had maximum r^2 of 0.10 and $x_{1/2}$ of 4.0 cM and 3.5 cM, respectively (**Figure 2-5a,c**). In mule deer, LG11 had slightly higher maximum r^2 (≈ 0.13) and a substantially slower rate of decline ($x_{1/2}$ = 5.3 cM; **Figure 2-5d**) than LG23, which had maximum r^2 of 0.12 and $x_{1/2}$ of 3.6 cM (**Figure 2-5b**).

2.3.6.2 Significant LD between Syntenic Markers

I tested the significance of composite LD between marker pairs using both the original and the pooled datasets in order to estimate the extent of LD and the effect of pooling rare alleles on test results. Given the substantial number of multiple comparisons involved, I indicated the levels of significance using both the scatter plots of raw *P*-values (**Figure 2-6**; **Figure 2-7Figure 2-6**) and the proportions of marker pairs in significant LD ($p < 0.05$) (**Figure 2-8**), rather than applying a Bonferroni-corrected significance level to each test.

Both species exhibited highly significant associations ($p \ll 0.001$) almost exclusively within a predicted distance of 1 cM (**Figure 2-6**; **Figure 2-7**). There were four marker pairs predicted to be within 1 cM in the white-tailed deer panel (**Figure 2-2**; BMS1669_URB021B on LG23; BM6445_UMBTL184 and BMS460_CP34 on LG11; DIK4009_DIK5136 on LG5), and all except for one pair (BMS460 _CP34) showed highly significant LD ($p \ll 0.001$) in both the original (**Figure 2-6a,b**) and the pooled datasets (**Figure 2-6c,d**). BMS460 _CP34 had no association in the original dataset (p = 0.14) and only a weak association when rare alleles were pooled (p = 0.02). In mule deer, the BMS460_CP34 pair was not included, and associations of the remaining pairs were also weaker than in white-tailed deer (**Figure 2-7**). The UMBTL184_BM6445 pair was highly significant when pooled ($p \ll 0.001$) but was less so in the original genotype dataset (p = 0.002). The DIK4009_DIK5136 pair was only weakly associated in both datasets (p = 0.01), while the URB021B_BMS1669 pair was non-significant in both datasets (original p = 0.22; pooled p = 0.09).

Although the highly significant associations disappeared beyond 1 cM, weaker associations were detected up to at least 5 cM in both species as revealed by the elevated proportions of marker pairs in significant LD ($p < 0.05$) (**Figure 2-8**). The background levels of LD, measured by the proportions of nonsyntenic pairs in significant LD, were equivalent to the Type I error rate ($\alpha = 0.05$) in both species in both datasets (the original and the pooled datasets, respectively: $p = 0.50$ and $p = 0.99$ in white-tailed deer; $p = 0.66$ and $p = 0.41$ in mule deer). Proportions of significant LD within 1 cM were substantially higher than the background level in both species in both datasets ($p < 0.001$). In white-tailed deer, statistically higher proportions extended up to 5–10 cM in the original dataset ($p = 0.002$ for 1–5 cM; $p < 0.001$ for 5–10 cM) and only up to 1–5 cM in the pooled dataset ($p < 0.001$) (**Figure 2-8a**). In mule deer, the elevated proportions were also observed up to 5–10 cM in both datasets, but only the 1–5 cM bin in the original dataset was statistically significant ($p < 0.001$) (**Figure 2-8b**), most likely due to the lack of power from having a smaller set of markers.

2.3.6.3 Effect of Pooling Rare Alleles

There was no noticeable shift associated with pooling of rare alleles in the overall patterns of the syntenic LD, while the statistical significance of some comparisons changed considerably (**Figure 2-6**; **Figure 2-7**). Probability (negative log-transformed) differences between the original and the pooled datasets ranged from -2.417 to 2.908 in white-tailed deer and from -1.076 to 1.922 in mule deer, indicating as extreme as 10–1,000 fold changes in *P*-values in both directions. Pooling had, however, no average effect in white-tailed deer ($p = 0.73$). In mule deer, there was a tendency for slightly decreased probability associated with pooling of rare alleles ($p = 0.045$). Nonsyntenic marker comparisons did not show significant mean difference before and after pooling in either white-tailed deer ($p = 0.649$) or mule deer ($p = 0.631$).

Extreme examples of the effects on individual comparisons include the following. BM3501_BMS460 pair at predicted distance of 12.2 cM in white-tailed deer (LG11; **Figure 2-2**) resulted in a loss of a highly significant association ($p = 0$ to $p = 0.008$) (**Figure 2-6b**, **d, data point A**). Contrarily, DIK4520_DIK4242 pair at predicted distance of 2.9 cM in white-tailed deer (LG23; **Figure 2-2**) had increased the association from $p = 0.01$ to highly significant ($p \ll 0.001$) when pooled (**Figure 2-6b**,**d, data point**

B). Overall, the effects of pooling were, however, in varying strengths and directions (increased or decreased significance of LD) and appeared unpredictable.

2.3.6.4 Significant LD between Distant Markers

Few distant marker pairs were in highly significant LD ($p \ll 0.001$). In white-tailed deer as mentioned previously, the highly significant LD in BM3501_BMS460 pair over 10 cM predicted distance (**Figure 2-6b, data point A**) disappeared after the rare alleles were pooled (**Figure 2-6d, data point A**). The only exception was ILSTS086_BMS1669 pair in mule deer at predicted distance of 4.6 cM (LG23; **Figure 2-3**), where the association was highly significant regardless of the pooling of rare alleles (**Figure 2-7b,d data point A**). There were no highly significant associations between nonsyntenic markers in either species.

2.4 Discussion

The present study investigated the feasibility of association mapping in CWD-affected wild deer populations. I amplified bovine microsatellite markers from the target LGs to determine the rate of cross-species amplification and construct the marker panels. I then tested the presence of population structure in the CWD-affected white-tailed deer and mule deer populations to evaluate its potential consequences for association studies. Finally I characterized the levels of LD to estimate the required marker density for association mapping. These findings combined allowed me to assess the feasibility of the comparative approach and the suitability of my target populations for association mapping, thereby illuminating the future prospects of the genomic research in indentifying CWD risk factors.

2.4.1 Cross-species Amplification and Marker Panel

The amplification of bovine markers in deer traverses a fairly large evolutionary distance between the families Bovidae and Cervidae with the divergence time of about 30 million years ago (MYA) (HASSANIN and DOUZERY 2003; FERNANDEZ and VRBA 2005). My polymorphic success rate of bovine markers in white-tailed deer was 27.9%, lower than some previous estimates that were over 50 % (KÜHN *et al.* 1996; SLATE *et al.* 1998). The discrepancy is likely due to differences in PCR and fragment analysis protocols,

levels of optimization effort, and sample sizes used to test genotyping reliability. Furthermore, the selective use of well-tested markers in previous studies has likely contributed to the higher success. Based on the systematic marker sampling, moderate optimization and thorough screening, my amplification rate is probably representative for markers from the high density bovine microsatellite map (IHARA *et al.* 2004). Thus, the large number of amplified loci that were subsequently found to be affected by allelic drop out and other genotyping difficulties (**Figure 2-1**; **Table 2-2**) is likely due to the large evolutionary distance between cattle and white-tailed deer. Finally, I observed an unexpectedly low cross-species amplification (60.0%) from white-tailed deer to mule deer, which are two closely related species with the estimated divergence time of ~1–5 MYA (FERNANDEZ and VRBA 2005; GILBERT *et al.* 2006). This could be related to the fact that the primers were bovine origin because it implies primer-template sequence divergence is larger in general, including amplifying markers. This could explain why the considerable number of markers which had passed the screening did not reliably amplify across the large population sample in both species.

My cross-amplification of 215 bovine microsatellites yielded 60 polymorphic loci in white-tailed deer that are predicted to be on three LGs (**Figure 2-2**). The mean predicted interval was 6.26 cM (**Table 2-4**), which is only slightly higher than the first generation, low density bovine microsatellite maps (BISHOP *et al.* 1994; MA *et al.* 1996). The panel, however, still contains two to three times the microsatellite density of the current red deer genetic map (SLATE *et al.* 2002). Despite the large mean interval, there were four marker pairs at distances of less than 1 cM, owing to the increased marker sampling in the candidate regions (**Figure 2-2**). Not only does this illustrate the potential for a higher resolution map with the screening of additional bovine markers, these marker pairs are also expected to be within distances for significant LD. On the other hand, both the white-tailed deer and the mule deer panels contained large gaps over 20 cM, especially in some parts of LG11 and 5 (**Figure 2-2**; **Figure 2-3**), where amplification failure rates were high. I expect these gaps can be filled by sampling and screening additional bovine markers unless there had been a large scale deletion of the chromosomal segments. Overall, the density and the distribution of markers on the current panels are likely too sparse and incomplete to offer LG-wide coverage.

My cross-amplification rate (27.9 %) provides an estimate of the maximum density that could be achieved using the high density bovine map. Because the target

bovine LGs contained a total of 535 microsatellites, of which only 215 were screened this time, nearly 70 more markers could potentially be incorporated into the white-tailed deer panel upon further screening. The density of this map would be comparable to the medium density bovine map (KAPPES *et al.* 1997) with the average interval of around 3.4 cM. The deer map could be improved by adding ovine and caprine microsatellites (VAIMAN *et al.* 1996; MADDOX *et al.* 2001) that are expected to transfer to cervids at similar rates as cattle, or possibly higher (ENGEL *et al.* 1996; RØED 1998), as well as a suite of anonymous (i.e. not mapped) microsatellites isolated from various cervid species (e.g. DEWOODY *et al.* 1995; WILSON *et al.* 1997). If large scale microsatellite linkage mapping were to be undertaken for a deer species, the cross-species amplification of the existing ungulate markers has the potential to produce a genetic map containing > 1,000 markers across the genome at a mean interval potentially as small as 2–3 cM. A genetic map of this resolution could certainly enable fine-scale linkage mapping of CWD and various other traits of evolutionary and commercial importance, but whether it would be sufficient for association mapping will depend on the levels of LD in target populations.

2.4.2 Population Structure

Cryptic population structure, or the presence of distinct subgroups with varying allele frequencies, can cause significant LD between unlinked loci as well as spurious associations between markers and disease phenotypes if the disease prevalence differs among the subgroups (e.g. LANDER and SCHORK 1994). Therefore, discerning population structure is a necessary step in studies of LD and association mapping. In both my target populations the significant positive F_{IS} (**Table 2-2; Table 2-3**) indicated weak genetic substructure, but the Bayesian clustering analyses failed to detect distinct genetic clusters within either sample (**Figure 2-4**). This pattern of weak differentiation at the landscape level without strong evidence of subdivisions is consistent with previous reports for white-tailed deer and mule deer populations in areas overlapping with the present study, where very weak but significant positive F_{ST} were found among pre-assigned subpopulations (0.0032, BLANCHONG *et al.* 2008; 0.0086; NAKADA 2009).

Weak differentiation in the absence of major barriers to gene flow can be driven by patterns such as isolation by distance (WRIGHT 1943) and polygamous mating systems with female philopatry typical of mammals (CHESSER 1991). The isolation by distance patterns across continuous landscapes have been demonstrated in deer populations and is

known to result from limited dispersal distance due to social factors (PURDUE *et al.* 2000; NAKADA 2009). Moreover, in white-tailed deer like most mammals, philopatric females form small social groups consisting of highly related individuals, while males disperse and maintain gene flow, resulting in a fine-scale genetic structure within populations (MATHEWS and PORTER 1993; COMER *et al.* 2005; MILLER *et al.* 2010). GREAR *et al.* (2010) and NAKADA (2009), in fact, have identified this fine-scale pattern in white-tailed deer and mule deer populations from the respective areas investigated in the present study, where they found elevated levels of relatedness among females in close proximity at a scale of 3–4 km.

In summary, the lack of population subdivision despite the weak genetic differentiation in my samples is consistent with the evidence of social structure provided by the previous landscape genetic studies. This suggests LD analyses and association studies in these populations are unlikely to severely suffer from spurious associations. However, the presence of fine-scale structure suggests cautions need to be taken in association studies to minimize the effect of varying levels of relatedness. That is, spurious associations might be observed if positive samples are biased in related females that tend to share more common alleles. Careful selection of matched case-control samples using fine-scale geographic information could help avoid this bias (discussed further in **Chapter 3**).

2.4.3 Linkage Disequilibrium

2.4.3.1 Extent and Patterns of LD

There was a sharp decline of LD with predicted distance in both white-tailed deer and mule deer regardless of the metrics (i.e. composite r^2 or genotypic association) or the datasets (i.e. pooled or not). This indicates that the large-scale marker order of the bovine map is roughly conserved in deer. However, I also found that the predicted distance explained only a small fraction of the variation and that there was an apparent heterogeneity among the LGs in the patterns of decline at a scale of approximately 0–5 cM (**Figure 2-5**). One potential source of variance is the deviation of actual marker positions from the bovine reference positions, which is not unexpected based on the long evolutionary separation between the two lineages. Another likely cause is the spatial complexity of LD. At fine-scales, high resolution LD studies in humans and domestic

species revealed extensive haplotype blocks often ranging from a few to over 100 kb, segmented by recombination hotspots stretching across ~ 2 kb (e.g. WALL and PRITCHARD 2003; LINDBLAD-TOH *et al.* 2005; VILLA-ANGULO *et al.* 2009). Thus, depending on the location of these structures, the levels of LD between markers would vary beyond what are expected as a function of the predicted distance. At chromosomal-scale, large differences in recombination rates are well known in humans (KONG *et al.* 2002). Significant inter-chromosomal heterogeneity in the observed levels of LD are also reported in other animals, but issues of low marker density and large variability of LD metrics appear to remain as likely causes, particularly for studies that employed low density microsatellites (SLATE and PEMBERTON 2007; LI and MERILA 2010). Since my study investigated only three LGs and relatively sparsely spaced markers, detailed inferences from the observed spatial variations are limited.

I found higher than background levels of LD to extend beyond 1 cM and potentially > 5cM, based on the composite r^2 ($x_{1/2}$) and the proportions of marker pairs in significant LD (**Figure 2-5**; **Figure 2-8**). These levels of association are, however, expected to be too weak to be useful for association mapping. This is because at 1–5 cM only ~20 % of the marker pairs were in significant LD at the nominal level ($p < 0.05$) with few of them highly significant ($p \ll 0.001$). The highly significant associations were instead largely restricted to <1 cM (**Figure 2-6**; **Figure 2-7**), where I had only four and three marker pairs in white-tailed deer and mule deer panels, respectively. This resulted in the inability of my marker panels to better characterize the extent of useful LD at the fine scale (≪1 cM) and may have led to the low composite r^2 values (< 0.15) even at 0 cM predicted distance (**Figure 2-5**). Because r^2 is the correlation coefficient that measures the amount of information one locus (its *genotypes* for the composite measures) provides about the other locus (reviewed in ARDLIE *et al.* 2002), my results suggest even the supposedly closely linked markers do not provide useful levels of LD for association studies. We should note, however, that composite LD measured from microsatellite genotypes has statistical properties that are not as easily interpretable as standard measures for SNP-based haplotype data, and I will discuss this issue in the following section (**Section 2.4.3.2**).

The weak overall LD (≪1 cM) in the deer populations means that dense SNP panels will be required in the future to characterize the extent of short-range LD in the orders of tens to hundreds of kilobases. Such weak levels of LD are known to be typical

in humans, where useful LD (SNP $r^2 \approx 0.3$) extends only ~10–30 kb in European and Asian populations and < 5 kb in African populations (ARDLIE *et al.* 2002; WEISS and CLARK 2002; ALTSHULER *et al.* 2005; FRAZER *et al.* 2007). Short-range LD under ~100 kb are also found in wild mice (*Mus musculus domesticus*) (LAURIE *et al.* 2007) and outbreeding populations of wolves (*Canis lupus*) and coyotes (*C. latrans*) (GRAY *et al.* 2009) as well as some domestic animals, including Chinese pigs (*Sus scrofa*) (AMARAL *et al.* 2008) and a variety of cattle breeds (MCKAY *et al.* 2007; VILLA-ANGULO *et al.* 2009; QANBARI *et al.* 2010). Apart from variability due to the choice of metric, livestock populations typically show higher levels of LD because of their demographic history involving selection, bottlenecks, admixture and/or isolation which lead to small effective population sizes (e.g. FARNIR *et al.* 2000; SUTTER *et al.* 2004; AMARAL *et al.* 2008). For instance, LD over 100 kb and even above 1 Mb (cM) have been reported in various dog breeds (*C. lupus*) (SUTTER *et al.* 2004; LINDBLAD-TOH *et al.* 2005; GRAY *et al.* 2009), horses (*Equus caballus*) (WADE *et al.* 2009), European pigs (AMARAL *et al.* 2008), and chickens (*Gallus gallus*) (ANDREESCU *et al.* 2007).

Among wild animals, long-range LD have been found in inbred wolf populations (GRAY *et al.* 2009), collared flycatcher (*Ficedula albicollis*) (BACKSTRÖM *et al.* 2006), and bighorn sheep (*O. canadensis*) (MILLER *et al.* 2011). Even more extensive LD over tens of centimorgans have been reported in populations of red deer (SLATE and PEMBERTON 2007) and Siberian jays (*Perisoreus infaustus*) (LI and MERILA 2010). In particular, I found, as I expected, much weaker LD in my white-tailed deer and mule deer populations compared to the red deer population studied by SLATE and PEMBERTON (2007). While also using microsatellite markers, SLATE and PEMBERTON (2007) observed long-range LD even between nonsyntenic markers, which they showed to be best explained by recent admixture due to the introduction of a reproductively successful male into the island population. In contrast, the absence of significant LD between distant (> 5–10 cM) and nonsyntenic marker pairs in my populations is consistent with the lack of strong population structure (**Figure 2-4**) and the high levels of genetic diversity in my samples (**Table 2-2; Table 2-3**). Moreover, it agrees with the species' semi-continental distributions in North America with consistently high genetic diversity across most areas, evidently achieved by high dispersal capability and habitat generality of the species (e.g. VAN DEN BUSSCHE *et al.* 2002; DEYOUNG *et al.* 2003; LATCH *et al.* 2009).

2.4.3.2 Measures of LD

It has been repeatedly pointed out that the choice of LD measures can have substantial effects on the observed levels of LD (e.g. PRITCHARD and PRZEWORSKI 2001; ZHAO *et al.* 2005). In humans and model organisms SNP-based physical maps largely replaced microsatellite-based genetic maps, and r^2 became the common standard to determine the extent of useful LD for association studies (ARDLIE *et al.* 2002; WEISS and CLARK 2002; ALTSHULER *et al.* 2005). In non-model organisms, however, microsatellites are still widely employed (e.g. ZHAO *et al.* 2005; SLATE and PEMBERTON 2007; ZHAO *et al.* 2007), and LD measures based on multiallelic genotypes are known for some statistical issues that can make interpretations difficult. Firstly, microsatellite-based LD estimates may be inflated relative to SNPs because of the large number of alleles and higher mutation rates (reviewed in PRITCHARD and PRZEWORSKI 2001; SLATKIN 2008). Empirical studies have shown that multiallelic *D'* was particularly problematic (e.g. MCKAY *et al.* 2007; LIPKIN *et al.* 2009), whereas simulations have shown that other multiallelic measures including r^2 are good predictors of biallelic LD (ZHAO *et al.* 2007). Secondly, microsatellites lack a standardized measure to represent LD among multiple pairs of alleles because none of the formulae for extending common measures (*D'*, r^2, and χ^2) to multiallelic situations are independent of allele frequencies (HEDRICK 1987; ZHAO *et al.* 2005). There are also a variety ways of standardizing across multiple alleles: for example, ZHAO *et al.*(2007) tested two different extensions of r^2, both of which differed from that of ZAYKIN *et al.*(2008) used here. Although I chose r^2, which has proven to be more robust to small sample sizes and allele frequencies, particularly rare alleles (ARDLIE *et al.* 2002; WEISS and CLARK 2002; ZHAO *et al.* 2005), the underperformance of the multiallelic r^2 remains an issue.

Genotype-based composite measures of LD were also problematic. While this approach is useful for avoiding errors through the estimation of haplotype frequencies or phases, its interpretation can be confounded by departure from HWE (**Eq.2-4**) (WEIR 1979). In addition, there is an expected loss of power compared to cases where haplotype information is available (PRITCHARD and PRZEWORSKI 2001; SLATKIN 2008). BARNAUD *et al.* (2006) found r^2 estimated from reconstructed haplotypes to be slightly higher than composite r^2 calculated from unphased genotypes in grape vine (*Vitis vinifera*), though they demonstrated the two measures corresponded well. Therefore, the low overall range of the composite r^2 in my samples (< 0.15; **Figure 2-5**) may be related by some degree to

the effects of HWD and reduced power. Unfortunately, however, haplotype frequency estimation or phase reconstruction procedures were unpractical for my data because of the number of microsatellite alleles, small sample sizes, and departure from HWE (**Table 2-2; Table 2-3**). Reliable estimation of haplotype phases would require larger sample sizes and preferably high density SNP genotypes, but would more accurately quantify LD.

The treatment of rare alleles was another important issue in estimating LD because it could skew the statistical measures and introduce errors in association tests. When SNPs are used, loci that do not meet minor allele frequency (MAF) set by individual studies are discarded (e.g. MAF = 0.1, BACKSTRÖM *et al.* 2006; 0.2, ANDREESCU *et al.* 2007; 0.05, LAURIE *et al.* 2007). When microsatellites are used, the problem could be more pronounced because more loci will have rare alleles and cannot all be discarded. Some studies pooled rare alleles below certain frequencies (e.g. 0.05, ABECASIS *et al.* 2001), but many other did not explicitly address this issue (e.g. FARNIR *et al.* 2000; MCRAE *et al.* 2002; SLATE and PEMBERTON 2007; LI and MERILA 2010). Because of the high genetic diversity of my target populations (**Table 2-2; Table 2-3**), I pooled rare alleles with frequencies of less than 0.05 for testing association between loci. As expected, pooling caused substantial changes in the probabilities of genotypic associations in some cases, demonstrating that false positive and negative conclusions could be reached by not accounting for abundant rare alleles (**Figure 2-6, data point A and B**). In addition, I observed an overall decrease in the probability of association in mule deer and fewer pairs of markers in significant LD ($p < 0.05$) in both species (**Figure 2-8**). These results together illustrate the risk of overestimating LD by not accounting for rare alleles.

2.5 Conclusion

Under the goal of assessing the feasibility of association mapping for CWD in wild deer, the present study produced novel insights to the comparative genetics of artiodactyls and the extent of LD in wild populations of large mammals with high dispersal capability. The cross-species amplification of bovine microsatellites was less efficient than some previous estimates, and the resulting maker panels were still of low density despite significant improvement from the existing red deer map. The absence of genetic structure and weak LD ($\ll 1$ cM) suggests these populations would be suitable for disease

association studies and fine-scale mapping of causative variants. An interval of 1 cM in the human genome, for example, can narrow the search down to ~10 genes on average (calculated from 26,000–38,000 genes on 2,907 cM or Mb in VENTER *et al.* 2001). Moreover, the levels of LD in humans (<100 kb) (REICH *et al.* 2001; ARDLIE *et al.* 2002; WEISS and CLARK 2002) are expected to enable the identification of single causative genes, if not the causative variants themselves. Such fine-scale mapping, thus, could offer a great advantage over the traditional family-based linkage mapping that usually results in broad QTL regions over 10 cM, spanning across hundreds of genes. The weak LD I found in deer, however, indicates significantly denser markers are required for coverage and necessitates the characterization of fine-scale LD (<100 kb), which I could not achieve using my marker panels derived from the bovine genetic map.

Based on the estimate of the cross-species amplification rate, the high density bovine microsatellite map will not be able to provide enough resolution for association mapping, even after an exhaustive transfer of the remaining bovine microsatellites not tested in this study. For instance, if I suppose useful LD of 10–100 kb, my current panel of 60 markers in white-tailed deer would only cover 0.6–6.0 cM (Mb), or 0.14–1.4 % of the > 440-cM total predicted length of the four bovine LGs. The maximum use of all available bovine microsatellites would only improve the coverage by ~ 2.5 times. Use of ~ 1,500 microsatellite markers genome-wide, an attainable density in deer, may be sufficient for association mapping in certain breeds of domestic sheep where it is enabled by longer-range LD of ~2.5 cM (MEADOWS *et al.* 2008). For most other domestic animal populations, however, high density microsatellites clearly do not suffice. Instead, ~10,000–100,000 SNPs are required for genome-wide coverage (SUTTER *et al.* 2004; LINDBLAD-TOH *et al.* 2005; AMARAL *et al.* 2008; WADE *et al.* 2009). In humans and other domestic populations with even more limited LD, roughly as many as ~300,000 – 500,000 SNPs are the minimum requirement (ALTSHULER *et al.* 2005; MCKAY *et al.* 2007; AMARAL *et al.* 2008). There is, therefore, a need for substantive genomic resources in deer, which includes a whole-genome sequence, a SNP map, and empirical determination of fine-scale LD.

With advancing high throughput sequencing technologies over the past few years, the genome sequencing of many non-model organisms may provide unprecedented tools for evolutionary and ecological analyses (SLATKIN 2008; ROKAS and ABBOT 2009). In cervids, red deer would likely be the first target for concerted genome sequencing and

SNP discovery projects given their importance in hunting and farming industries worldwide. Completion of such projects would provide not only the platform for the characterization of genome-wide LD and association studies but also an enhanced genomic reference for other cervid species. Furthermore, since elk are impacted by CWD in wild (SIGURDSON and AGUZZI 2007), the development of genomic resources may lead to a new age of CWD research by whole-genome association studies. The direct applicability of red deer genomic resources (e.g. a SNP array) to white-tailed deer or mule deer, however, is unlikely to be very high, based on recent findings of limited cross-species utility of bovid SNPs (SECHI *et al.* 2010). For example, studies found less than 2–3 % (1,000-1,500 SNPs) polymorphic rates of ~50,000 bovine and ovine SNP arrays in closely related wild species (estimated divergence of 5.8–6.8 MYA) (MILLER *et al.* 2011; PERTOLDI *et al.* 2010). Thus, based on the deep divergence of the red deer and the *Odocoileus* lineages over 10–20 MYA (FERNANDEZ and VRBA 2005; GILBERT *et al.* 2006), I would expect that species-specific genomic resources will be required for attaining enough SNP density for a genome-wide study.

Therefore, while awaiting the development of specific genomic resources, the identification of genetic risk factors for CWD in white-tailed deer and mule deer must rely on family-based linkage mapping or the candidate gene approach. Fortunately these approaches are tractable in the future because: 1) there are likely to be >1,000 mapped bovine microsatellites that will be informative in deer; and 2) for the candidate gene approach, transcriptome sequencing of white-tailed deer has been completed very recently on a next-generation sequencing platform, producing ~10,000 SNPs in >5,000 expressed genes that could be used to interrogate many candidate genes (MALENFANT, *et al.* unpublished data). In conclusion, the present study illuminated the challenges that most wild species face in obtaining sufficient genomic resources needed for conducting powerful genome-wide studies to address important medical and evolutionary questions. Given the huge socioeconomic and human health implications of CWD, the development of genomic resources for cervids should be a high priority.

2.6 Figures and Tables

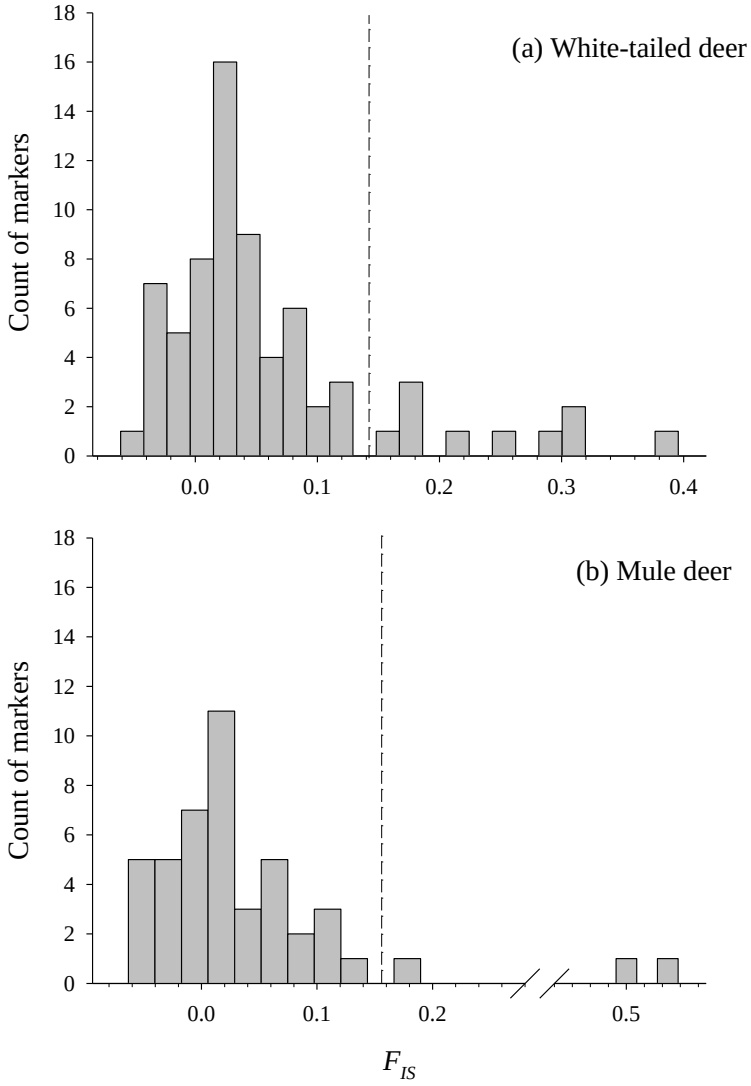

Figure 2-1 F_{IS} distributions of (a) 70 microsatellite markers in white-tailed deer (N = 184) and (b) 45 markers in mule deer (N = 192). Dashed lines indicate the natural breaks (roughly corresponding to one standard deviation from the mean) used to identify the outlying loci with high F_{IS} . These loci were excluded from the subsequent analyses.

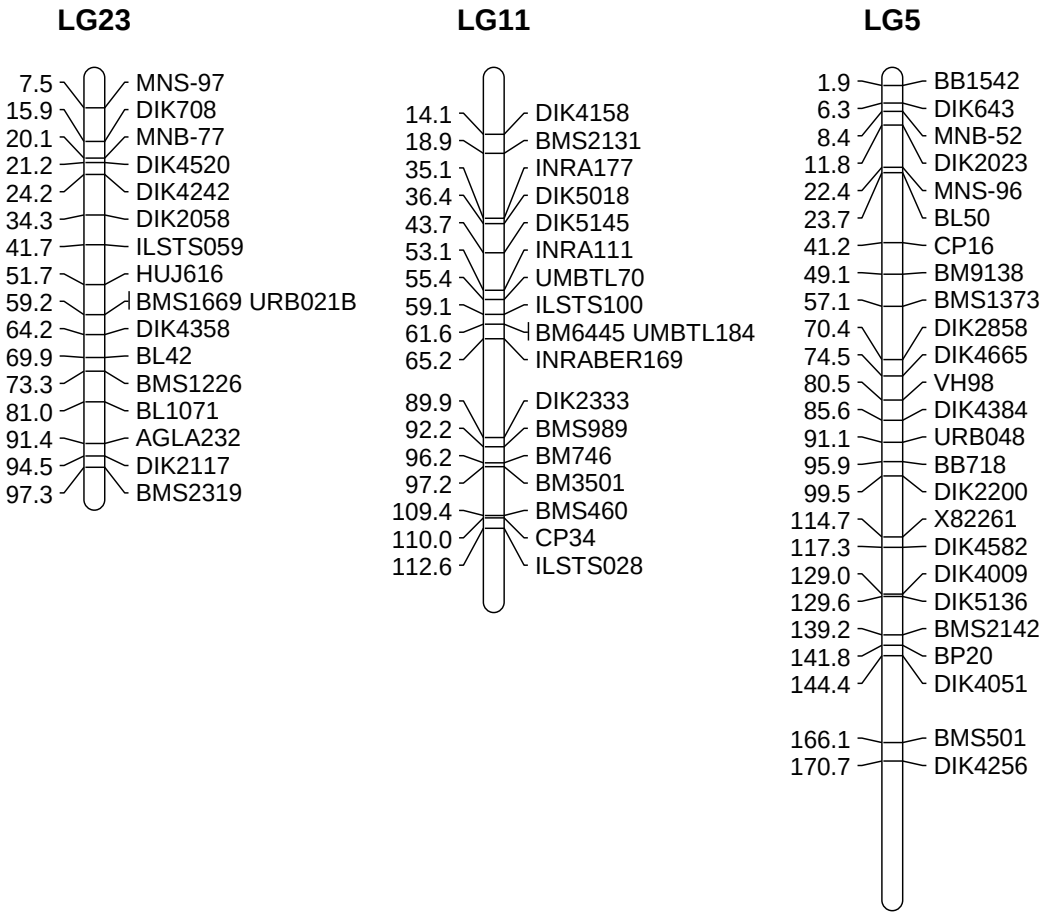

Figure 2-2 Predicted positions (cM) of the 60 bovine microsatellite markers included in the white-tailed deer panel. The markers are expected to belong to three red deer LGs (LG23, 11, and 5) selected as candidates harboring CWD-associated regions (**Table 2-1**). Marker positions were inferred from the high density bovine genetic map (IHARA *et al.* 2004).

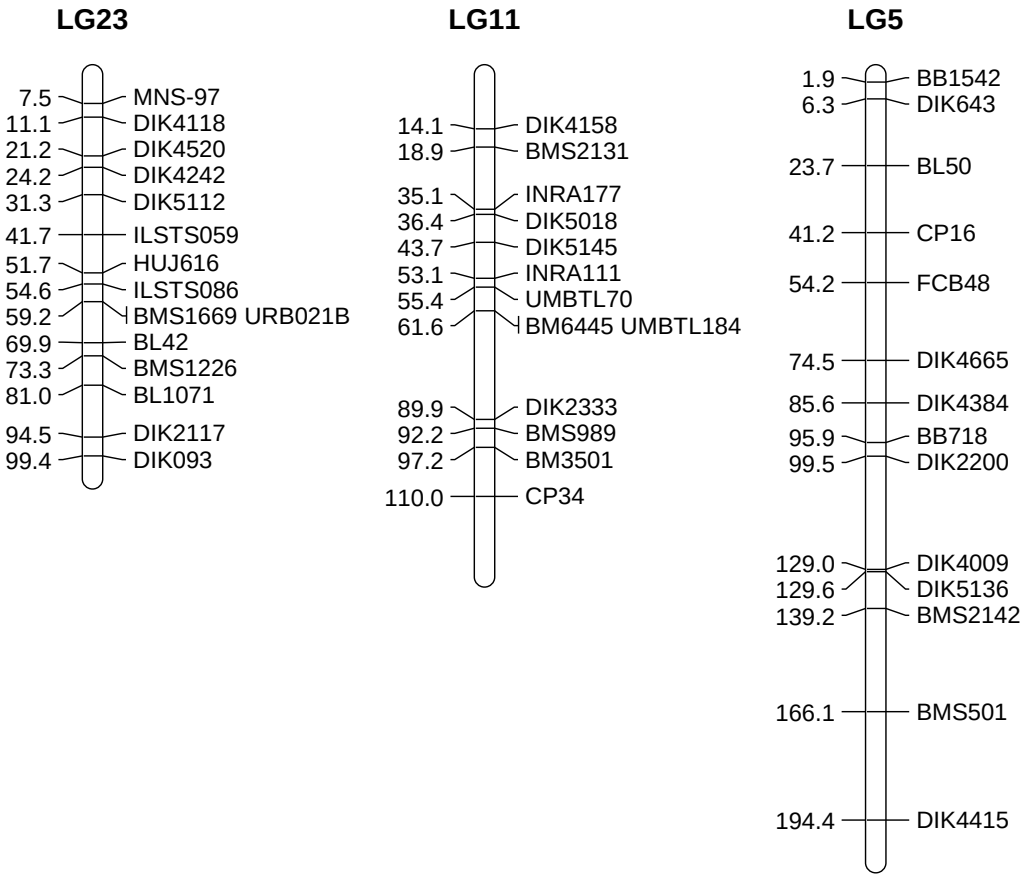

Figure 2-3 Predicted positions (cM) of the 42 bovine microsatellite markers included in the mule deer panel. The markers are expected to belong to three red deer LGs (LG23, 11, and 5) selected as candidates harboring CWD-associated regions (**Table 2-1**). Marker positions were inferred from the high density bovine genetic map (IHARA *et al.* 2004).

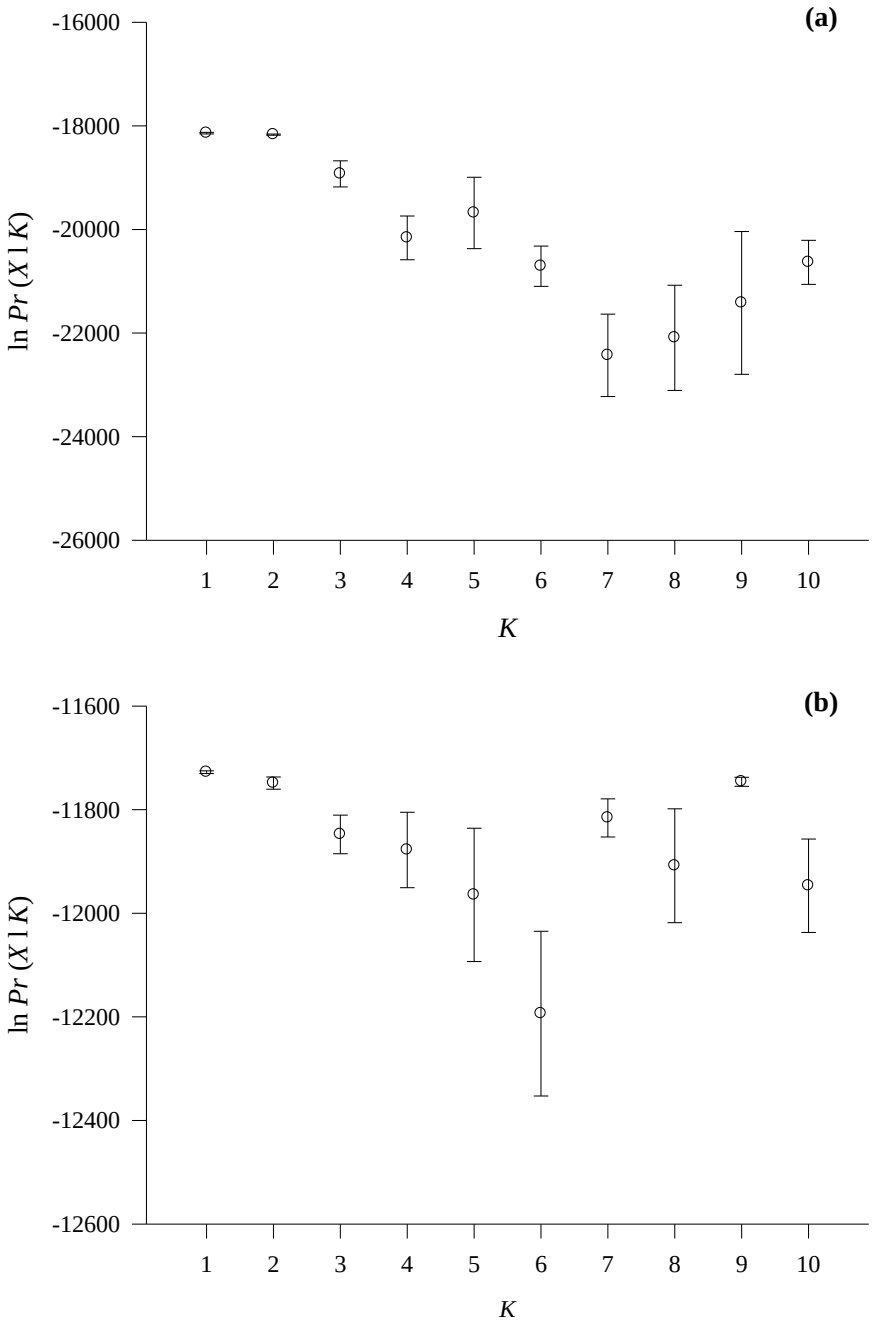

Figure 2-4 Mean posterior probability (ln $Pr(X|K)$ ± standard error) for each pre-assigned number of subpopulations (*K*) obtained via the Bayesian clustering method implemented in Structure v2.2.3. Thirty loci in white-tailed deer (a) and 22 loci in mule deer (b) that were not in significant LD with another locus ($p < 0.05$) were included in the analyses. Overall, no structure was detected in either samples as indicated by the highest posterior probabilities at $K = 1$.

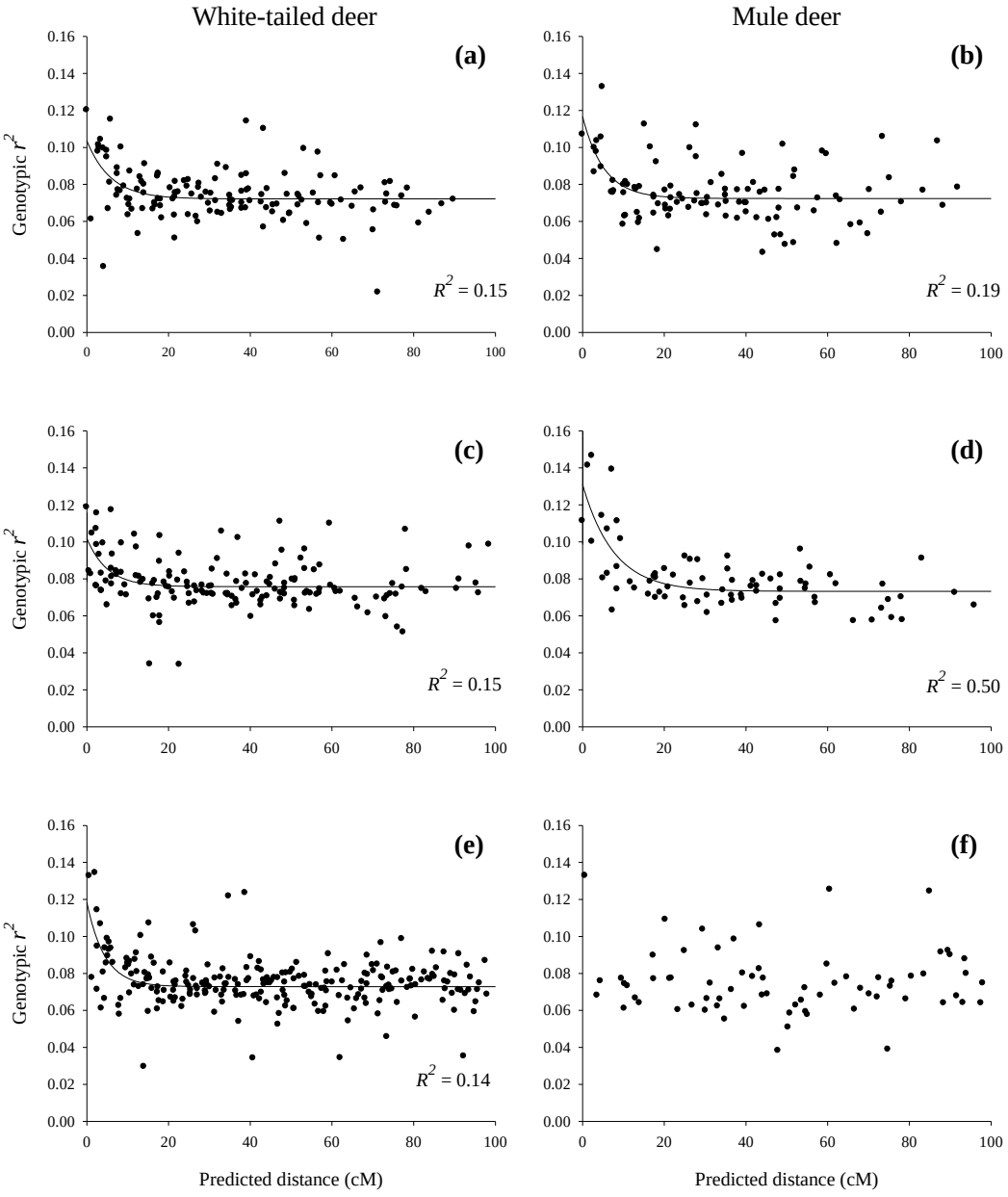

Figure 2-5 Composite r^2 between syntenic marker pairs as a function of predicted marker distance (cM) for red deer LGs 23 (a, b), 11 (c, d), and 5 (e, f) in white-tailed deer and mule deer. The marker distances were inferred from the bovine map (IHARA *et al.* 2004). Exponential decay regression lines ($y = y_0 + a\, e^{-bx}$) were fitted, and all coefficients tested significant ($p \ll 0.05$) except for LG5 in mule deer (f).

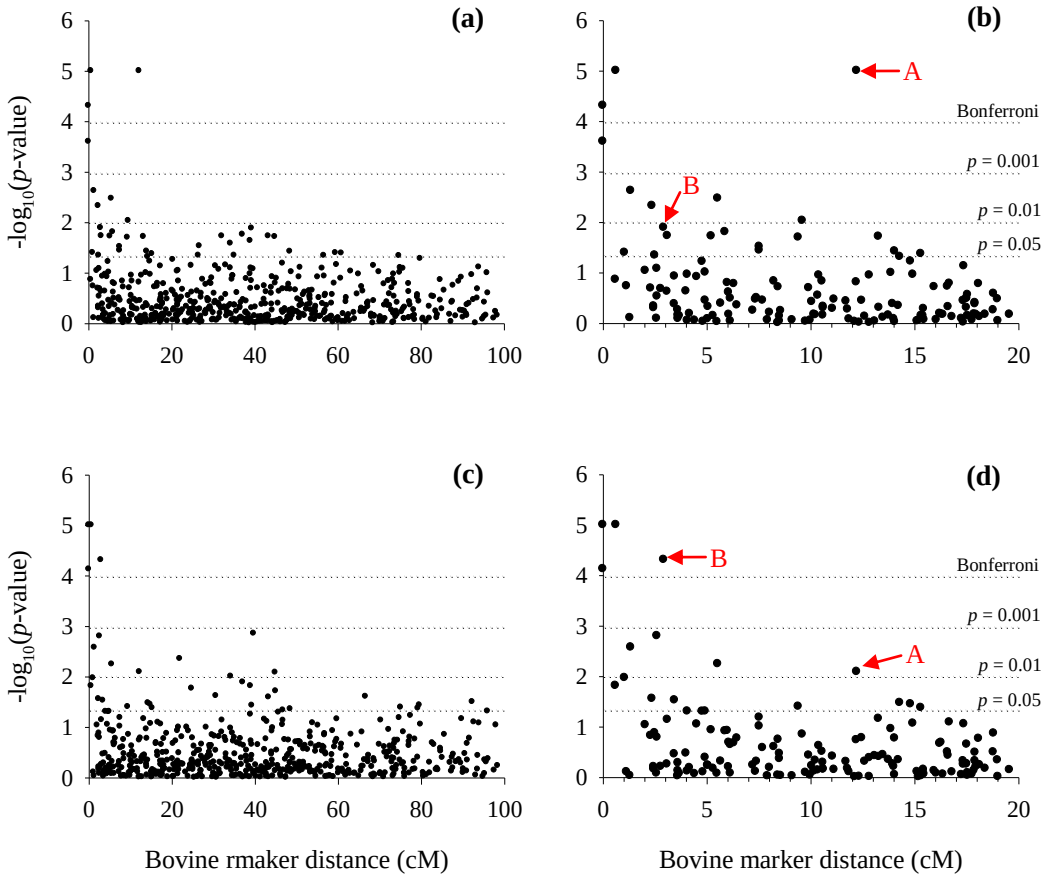

Figure 2-6 Probability of genotypic associations for syntenic marker pairs as a function of predicted marker distance (cM) in white-tailed deer. The top panels (a, b) and the bottom panels (c, d) show the original and pooled datasets, respectively. The right panels (b, d) show the same data as the left panels (a, c), at a closer scale.

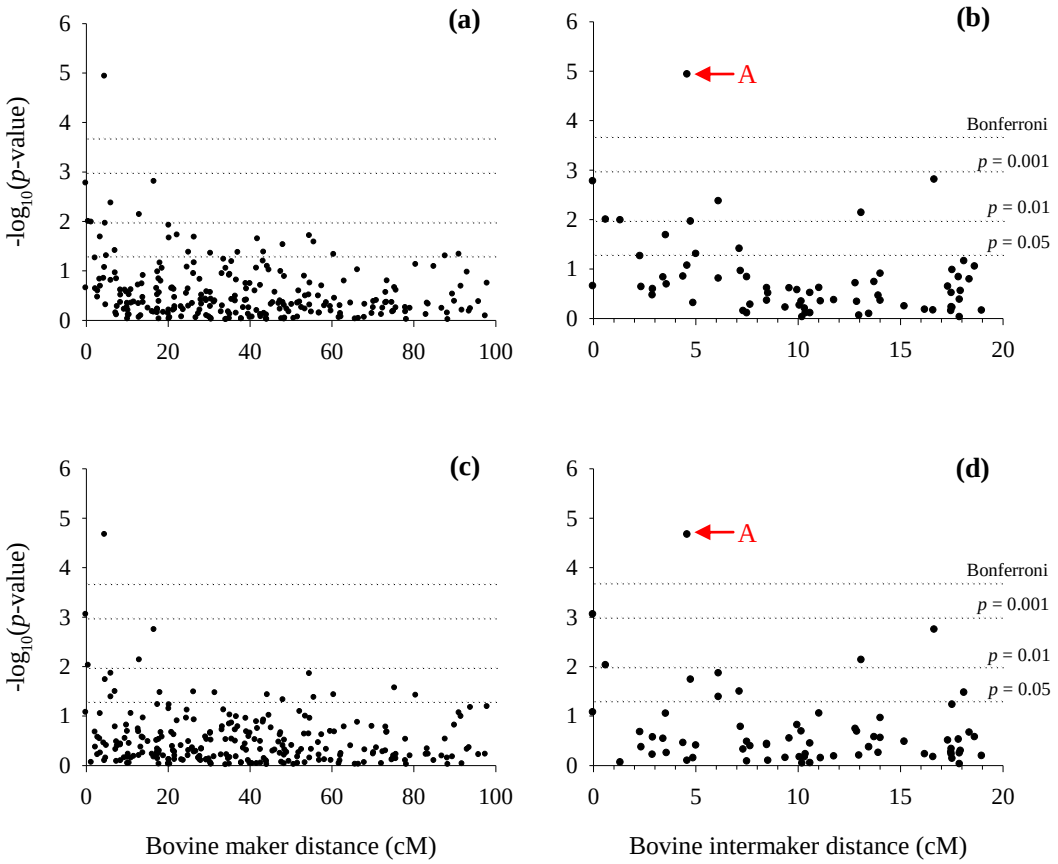

Figure 2-7 Probability of genotypic associations for syntenic marker pairs as a function of predicted marker distance (cM) in mule deer. The top panels (a, b) and the bottom panels (c, d) show the original and pooled datasets, respectively. The right panels (b, d) show the same data as the left panels (a, c), at a closer scale.

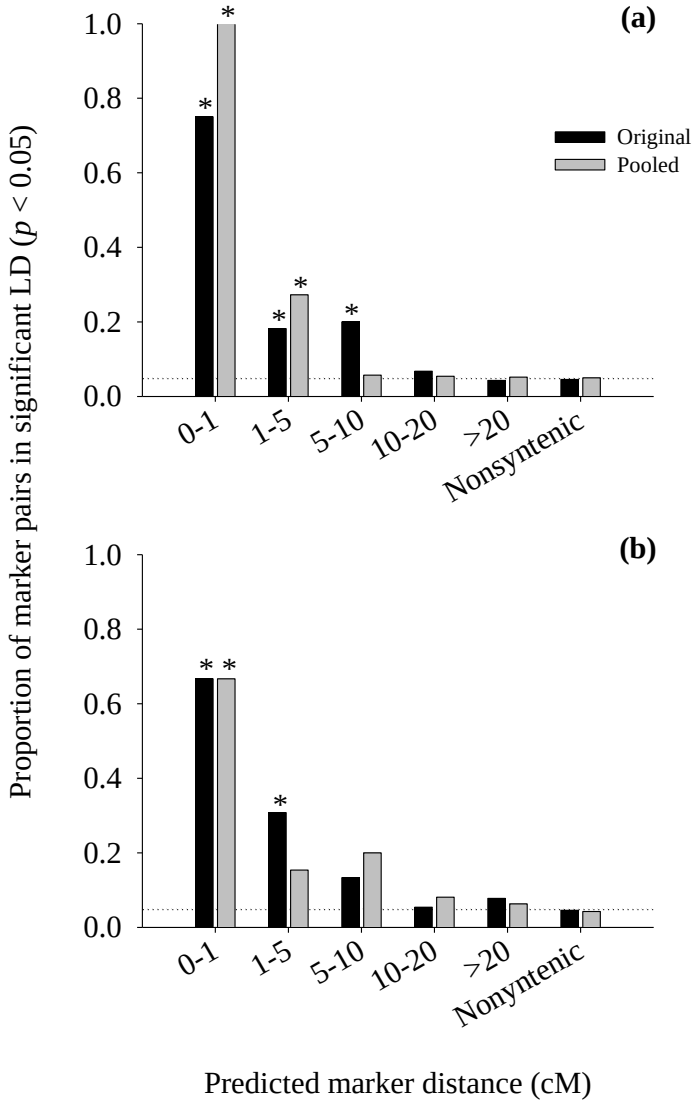

Figure 2-8 Proportions of marker pairs in significant LD ($p < 0.05$) summarized by the predicted marker intervals (cM) in white-tailed deer (a) and mule deer (b). Marker intervals (cM) were inferred from the bovine map (IHARA *et al.* 2004). Associations were tested using both the original genotype and the pooled genotype (rare alleles with frequency < 0.05 were pooled). Dotted lines indicate the Type I error rate at $\alpha = 0.05$. Proportions of significant LD in nonsyntenic marker pairs were statistically equivalent to the Type I error rate in both species using both datasets. * Indicates significant difference from the nonsyntenic proportions after the Bonferroni correction.

Table 2-1 List of candidate red deer LGs with the predicted assignment of candidate genes and QTL regions for TSEs.

Red deer LG	Cattle chromosome	Candidate gene and QTL	Tested species (chromosome) / disease phenotype	Reference
LG 23*	Bta 13*	*PRNP*	human, sheep, cattle, deer, etc. / TSE incubation period, susceptibility in experiments and in nature	Reviewed elsewhere
		Genome-wide suggestive QTL	cattle / BSE case-control	(ZHANG *et al.* 2004)
LG 11*	Bta 11*	*IL1B/IL1RN*	sheep (3)/ scrapie case-control	(MARCOS-CARCAVILLA *et al.* 2007)
		Genome-wide significant QTL	mice (2) / scrapie incubation period	(LLOYD *et al.* 2001)
		Genome-wide significant QTL	mice (2)/ BSE incubation period	(MANOLAKOU *et al.* 2001; LLOYD *et al.* 2002)
LG 5*	Bta 17*	Genome-wide significant QTL	cattle / BSE case-control	(ZHANG *et al.* 2004)
	Bta 19*	*NF1*	cattle / BSE case-control	(GELDERMANN *et al.* 2006)
		Genome-wide suggestive QTL	cattle / BSE case-control	(ZHANG *et al.* 2004)
		Genome-wide significant QTL	mice (11)/ scrapie incubation period	(STEPHENSON *et al.* 2000; LLOYD *et al.* 2001)
		Genome-wide significant QTL	mice (11)/ BSE incubation period	(LLOYD *et al.* 2002)
LG 13	Bta 21	*HSP90AA1*	sheep (18)/ scrapie case-control	(MARCOS-CARCAVILLA *et al.* 2008a)
		Genome-wide suggestive QTL	sheep (18)/ scrapie incubation period (natural)	(MORENO *et al.* 2008)
		Genome-wide significant QTL	mice (12)/ scapie ncubation period	(LLOYD *et al.* 2001)

Table 2-1 Continued.

Red deer LG	Cattle chromosome	Candidate gene and QTL	Tested species (chromosome) / disease phenotype	Reference
LG 15	Bta 26, 28	*SPRN*	mice (7)/ no association with scrapie incubation time	(LLOYD *et al.* 2009)
		Genome-wide significant QTL	mice (7)/ scrapie incubation period	(MORENO *et al.* 2003)
		Genome-wide suggestive QTL	mice (7)/ scrapie incubation period	(LLOYD *et al.* 2001)
LG 24	Bta 22	*RPSA*	sheep (19)/ scrapie case-control	(MARCOS-CARCAVILLA *et al.* 2008b)
		Genome-wide significant QTL	mice (9)/ scrapie incubation period	(STEPHENSON *et al.* 2000)
LG 6, 17	Bta 6	Genome-wide suggestive QTL	cattle / BSE case-control	(ZHANG *et al.* 2004)
		Genome-wide suggestive QTL	sheep (6)/ scrapie incubation period (natural)	(MORENO *et al.* 2008)
		Genome-wide suggestive QTL	mice (5)/ scrapie incubation period	(MORENO *et al.* 2003)

QTL mapped on mice chromosomes may not directly correspond to the indicated bovine chromosomes due to large confidence intervals around the QTL and complex chromosomal rearrangements between the two species.

* Cattle chromosomes and homologous red deer LGs selected for this study.

Table 2-2 PCR conditions, diversity statistics, and genotyping issues for the 70 bovine microsatellites in white-tailed deer (*N*=184).

Marker	Deer LG	Cattle LG	Position (cM)	Ta	M13 dye	Number of alleles	Size range (bp) ‡	*Ho*	*He*	F_{IS}	Possible sources of genotyping error §
MNS-97	23	13	7.5	52 °C	VIC	5	269-281	0.420	0.405	-0.0363*	
DIK4118 †	23	13	11.1	56 °C TD	PET	6	244-254	0.622	0.758	0.1797	stuttering; null
DIK708	23	13	15.9	60 °C TD	FAM	2	208-209	0.120	0.113	-0.0612	
MNB-77	23	13	20.1	54 °C TD	PET	3	219-224	0.268	0.301	0.1107	
DIK4520	23	13	21.2	54 °C TD	FAM	40	214-346	0.911	0.919	0.0083	
DIK4242	23	13	24.2	52 °C	FAM	14	266-306	0.863	0.862	-0.0020	
DIK5112 †	23	13	31.3	56 °C TD	FAM	11	186-219	0.650	0.821	0.2080**	null
DIK2058	23	13	34.3	52 °C	FAM	4	168-174	0.639	0.686	0.0676	
ILSTS059	23	13	41.7	52 °C	FAM	10	164-188	0.705	0.766	0.0804	null
HUJ616	23	13	51.7	52 °C	PET	9	124-142	0.825	0.814	-0.0132	
ILSTS086 †	23	13	54.6	52 °C	FAM	9	171-200	0.406	0.543	0.2534*	stuttering; null
BMS1669	23	13	59.2	52 °C	NED	13	109-141	0.792	0.842	0.0591	
URB021B	23	13	59.2	52 °C	NED	6	145-157	0.771	0.786	0.0186*	
DIK4358	23	13	64.2	54 °C TD	VIC	16	130-170	0.863	0.890	0.0295	
BL42	23	13	69.9	52 °C	VIC	9	252-270	0.777	0.801	0.0300	
BMS1226	23	13	73.3	50 °C	FAM	11	159-179	0.826	0.850	0.0288	
BL1071	23	13	81.0	54 °C TD	PET	16	182-212	0.821	0.836	0.0182	
AGLA232	23	13	91.4	50 °C TD	NED	3	162-164	0.364	0.396	0.0803	
DIK2117	23	13	94.5	52 °C	FAM	15	220-243	0.781	0.794	0.0163	
BMS2319	23	13	97.3	52 °C	PET	11	113-142	0.773	0.747	-0.0355	
DIK093 †	23	13	99.4	52 °C	NED	15	193-224	0.691	0.843	0.1811	null
DIK4158	11	11	14.1	52 °C	PET	17	235-275	0.911	0.889	-0.0247	

Table 2-2 Continued.

Marker	Deer LG	Cattle LG	Position (cM)	Ta	M13 dye	Number of alleles	Size range (bp) ‡	*Ho*	*He*	F_{IS}	Possible sources of genotyping error §
BMS2131	11	11	18.9	48 °C	PET	13	221-253	0.723	0.765	0.0555	
INRA177	11	11	35.1	52 °C	VIC	16	88-122	0.879	0.896	0.0184	
DIK5018	11	11	36.4	52 °C	NED	6	218-228	0.761	0.757	-0.0046	
DIK5145	11	11	43.7	54 °C TD	VIC	7	182-196	0.720	0.695	-0.0358	
INRA111	11	11	53.1	52 °C	PET	13	122-146	0.832	0.836	0.0051	
UMBTL70	11	11	55.4	54 °C TD	FAM	15	179-202	0.799	0.879	0.0910*	null
ILSTS100	11	11	59.1	50 °C TD	FAM	2	204-206	0.144	0.144	-0.0041	
BM6445	11	11	61.6	52 °C TD	VIC	13	127-155	0.842	0.856	0.0162	
UMBTL184	11	11	61.6	52 °C TD	PET	17	275-304	0.750	0.856	0.1243*	null
INRABER169	11	11	65.2	52 °C	NED	12	207-235	0.749	0.788	0.0502	
DIK2333	11	11	89.9	52 °C	PET	11	198-218	0.617	0.696	0.1136*	null
BMS989	11	11	92.2	52 °C	VIC	12	110-132	0.853	0.848	-0.0059	
BM746	11	11	96.2	52 °C	FAM	9	161-179	0.802	0.824	0.0264	
BM3501	11	11	97.2	56 °C TD	NED	16	168-206	0.806	0.825	0.0242	
BMS460	11	11	109.4	50 °C	NED	18	128-168	0.878	0.913	0.0388	
CP34	11	11	110.0	52 °C	FAM	10	117-143	0.776	0.810	0.0423	
ILSTS028	11	11	112.6	52 °C	VIC	3	150-154	0.087	0.085	-0.0341	
DIK5263 †	11	11	120.5	52 °C	PET	13	242-268	0.704	0.849	0.1714**	stuttering; null
BB718	5	17	0.0	52 °C	FAM	12	152-176	0.879	0.875	-0.0045	
URB048	5	17	4.8	52 °C	VIC	20	175-206	0.933	0.909	-0.0263	
DIK4384	5	17	10.3	52 °C	NED	13	222-246	0.885	0.888	0.0032	
VH98	5	17	15.4	52 °C	PET	11	172-200	0.798	0.826	0.0346	

Table 2-2 Continued.

Marker	Deer LG	Cattle LG	Position (cM)	Ta	M13 dye	Number of alleles	Size range (bp) ‡	*Ho*	*He*	F_{IS}	Possible sources of genotyping error §
DIK4665	5	17	21.4	52 °C	NED	12	199-229	0.824	0.835	0.0125	
DIK2858	5	17	25.5	54 °C TD	FAM	3	206-210	0.473	0.522	0.0948	
BMS1373	5	17	38.8	52 °C	VIC	7	115-121	0.566	0.588	0.0373	
FCB48 †	5	17	41.7	52 °C	NED	12	166-184	0.240	0.349	0.3122**	stuttering; null
BM9138	5	17	46.8	52 °C	NED	3	341-348	0.240	0.265	0.0930	
CP16	5	17	54.7	54 °C TD	VIC	16	92-128	0.851	0.861	0.0119	
BL50	5	17	72.2	52 °C	NED	10	227-247	0.802	0.827	0.0298	
MNS-96	5	17	73.5	52 °C	PET	3	179-187	0.072	0.076	0.0455	
DIK4383 †	5	17	78.1	52 °C	NED	4	225-235	0.491	0.710	0.3085**	stuttering; null
DIK2023	5	17	84.1	52 °C	PET	11	166-191	0.831	0.865	0.0393	
MNB-52	5	17	87.5	52 °C	FAM	2	185-187	0.398	0.411	0.0314	
DIK643	5	17	89.6	52 °C	VIC	6	177-187	0.747	0.767	0.0255	
BB1542	5	17	94.0	54 °C TD	PET	11	266-298	0.712	0.739	0.0372	
DIK2200	5	19	3.60	56 °C TD	NED	12	152-188	0.825	0.847	0.0259	
X82261	5	19	18.8	54 °C TD	VIC	4	258-264	0.650	0.709	0.0831	
DIK4582	5	19	21.5	52 °C	PET	5	221-235	0.564	0.581	0.0300	
DIK4009	5	19	33.1	54 °C TD	NED	15	196-224	0.891	0.904	0.0151	
DIK5136	5	19	33.7	48 °C TD	NED	10	260-278	0.724	0.715	-0.0125	
DIK5332 †	5	19	33.7	54 °C TD	VIC	2	241-244	0.306	0.428	0.2856**	null
BMS2142	5	19	43.3	52 °C	VIC	21	122-159	0.896	0.864	-0.0375	
BP20	5	19	45.9	52 °C	NED	3	225-227	0.363	0.387	0.0629	
DIK4051	5	19	48.5	52 °C	FAM	10	223-241	0.787	0.855	0.0804*	null

Table 2-2 Continued.

Marker	Deer LG	Cattle LG	Position (cM)	Ta	M13 dye	Number of alleles	Size range (bp) ‡	*Ho*	*He*	F_{IS}	Possible sources of genotyping error §
BMS501	5	19	70.2	52 °C	PET	16	136-172	0.786	0.861	0.0881*	null
DIK4256	5	19	74.8	52 °C	VIC	9	232-260	0.809	0.840	0.0373	
DIK4415 †	5	19	98.5	52 °C	NED	3	208-210	0.366	0.605	0.3956**	stuttering; null
DIK4898 †	5	19	109.6	52 °C	FAM	16	229-270	0.772	0.915	0.1565**	null
Mean						10.7		0.696	0.717	0.0289	
± SD						6.3		0.226	0.228	0.0417	

Reference: red deer LG assignments (SLATE *et al.* 2002); bovine marker position (IHARA *et al.* 2004); marker reference listed in IHARA *et al.* (2004)'s supplemental material at Genome Research website <http://genome.cshlp.org/content/14/10a/1987/suppl/DC1>.

H_O = observed heterozygosity; H_E = NEI (1978)'s unbiased gene diversity; F_{IS} = WEIR and COCKERHAM (1984)'s estimate; TD = touchdown PCR.

* Indicates significant deviation from HWE at nominal level ($\alpha = 0.05$); ** significant deviation from HWE after Bonferroni correction.

† Indicates markers with outlying F_{IS} (**Figure 2-1a**) and evidence of genotyping errors, thus, excluded from calculation of loci means and LD analyses

‡ Product sizes include 18 bp addition of M-13 primer sequence. Different weights of florescent dyes influenced the product size by 1-2 bp.

§ Micro-Checker (VAN OOSTERHOUT *et al.* 2004) was used to detect the possible presence of genotyping error under HWE assumption.

Table 2-3 PCR conditions, diversity statistics, and genotyping issues for the 45 bovine microsatellites in mule deer (*N* = 192).

Marker	Deer LG	Cattle LG	Position (cM)	Ta	M13 dye	Number of alleles	Size range (bp) ‡	*Ho*	*He*	F_{IS}	Possible sources of genotyping error §
MNS-97	23	13	7.5	54 °C	VIC	2	275 - 277	0.487	0.473	-0.0294	
DIK4118	23	13	11.1	56 °C	FAM	7	244 - 258	0.787	0.808	0.0253	
DIK4520	23	13	21.2	48 °C TD	FAM	11	217 - 237	0.767	0.760	-0.0098	
DIK4242	23	13	24.2	52 °C	VIC	11	278 - 300	0.846	0.857	0.0130	
DIK5112	23	13	31.3	52 °C TD	PET	6	201 - 215	0.431	0.429	-0.0052	
ILSTS059	23	13	41.7	52 °C	PET	4	169 - 175	0.704	0.678	-0.0386	
HUJ616	23	13	51.7	52 °C	FAM	5	125 - 139	0.619	0.592	-0.0460	
ILSTS086	23	13	54.6	52 °C	PET	16	173 - 207	0.847	0.843	-0.0054	
BMS1669	23	13	59.2	52 °C	FAM	15	110 - 144	0.926	0.889	-0.0421	
URB021B	23	13	59.2	52 °C	FAM	5	145 - 157	0.529	0.543	0.0255	
BL42	23	13	69.9	46 °C	NED	5	250 - 262	0.367	0.424	0.1355	null allele
BMS1226	23	13	73.3	48 °C	FAM	8	159 - 175	0.720	0.733	0.0187	
BL1071	23	13	81.0	54 °C TD	NED	3	186 - 190	0.173	0.166	-0.0382	
DIK2117	23	13	94.5	52 °C	VIC	10	223 - 241	0.767	0.780	0.0170	
DIK093	23	13	99.4	52 °C	FAM	9	193 - 217	0.702	0.794	0.1158*	null allele
DIK4158	11	11	14.1	48 °C	VIC	12	229 - 273	0.668	0.685	0.0237*	
BMS2131	11	11	18.9	48 °C	VIC	9	234 - 253	0.759	0.781	0.0277	
INRA177	11	11	35.1	52 °C	FAM	13	80 - 115	0.651	0.666	0.0231	
DIK5018	11	11	36.4	54 °C	NED	7	224 - 248	0.738	0.708	-0.0430	
DIK5145	11	11	43.7	54 °C TD	NED	7	180 - 192	0.798	0.772	-0.0336*	
INRA111	11	11	53.1	52 °C	FAM	7	129 - 147	0.644	0.718	0.1030*	null allele
UMBTL70	11	11	55.4	52 °C TD	NED	12	177 - 203	0.783	0.827	0.0537	

Table 2-3 Continued.

Marker	Deer LG	Cattle LG	Position (cM)	Ta	M13 dye	Number of alleles	Size range (bp) ‡	*Ho*	*He*	F_{IS}	Possible sources of genotyping error §
BM6445	11	11	61.6	52 °C TD	FAM	9	130 - 152	0.811	0.762	-0.0633	
UMBTL184	11	11	61.6	48 °C TD	VIC	8	271 - 291	0.782	0.796	0.0175	
DIK2333	11	11	89.9	52 °C	NED	7	197 - 206	0.696	0.717	0.0292	
BMS989	11	11	92.2	52 °C	FAM	9	112 - 134	0.676	0.705	0.0426	
BM3501	11	11	97.2	56 °C TD	NED	4	184 - 192	0.431	0.433	0.0054	
CP34	11	11	110.0	52 °C	FAM	5	115 - 129	0.453	0.486	0.0695*	
DIK5263 †	11	11	120.5	50 °C	FAM	7	234 - 249	0.370	0.448	0.1744**	stuttering; null allele
BB718	5	17	0.0	52 °C	PET	12	155 - 183	0.801	0.815	0.0176	
URB048 †	5	17	4.8	54 °C	NED	5	183 - 189	0.267	0.603	0.5575**	stuttering; null allele
DIK4384	5	17	10.3	52 °C	VIC	12	223 - 242	0.719	0.772	0.0695*	
DIK4665	5	17	21.4	52 °C	NED	6	206 - 216	0.723	0.707	-0.0217	
BMS1373 †	5	17	38.8	50 °C TD	PET	5	119 - 123	0.257	0.512	0.4994**	stuttering; null allele
FCB48	5	17	41.7	52 °C	PET	3	165 - 173	0.220	0.240	0.0852	
CP16	5	17	54.7	52 °C TD	FAM	6	104 - 118	0.638	0.713	0.1056	stuttering; null allele
BL50	5	17	72.2	52 °C	VIC	9	226 - 246	0.677	0.715	0.0532	
DIK643	5	17	89.6	52 °C	NED	5	181 - 189	0.665	0.667	0.0034	
BB1542	5	17	94.0	50 °C TD	PET	11	263 - 299	0.761	0.748	-0.0164	
DIK2200	5	19	3.6	56 °C TD	FAM	5	151 - 187	0.241	0.228	-0.0543	
DIK4009	5	19	33.1	52 °C TD	PET	3	200 - 204	0.374	0.380	0.0166	
DIK5136	5	19	33.7	52 °C TD	VIC	11	262 - 290	0.804	0.853	0.0574	
BMS2142	5	19	43.3	54 °C TD	FAM	5	130 - 148	0.382	0.394	0.0305	
BMS501	5	19	70.2	50 °C	PET	10	136 - 162	0.771	0.761	-0.0138	

Table 2-3 Continued.

Marker	Deer LG	Cattle LG	Position (cM)	Ta	M13 dye	Number of alleles	Size range (bp) ‡	*Ho*	*He*	F_{IS}	Possible sources of genotyping error §
DIK4415	5	19	98.5	52 °C	NED	4	207 - 210	0.497	0.546	0.0886	
Mean						7.8		0.639	0.65	0.0193	
± SD						3.5		0.19	0.19	0.0491	

Reference: red deer LG assignments (SLATE *et al.* 2002); bovine marker position (IHARA *et al.* 2004); marker reference listed in IHARA *et al.* (2004)'s supplemental material at Genome Research website <http://genome.cshlp.org/content/14/10a/1987/suppl/DC1>.

H_O = observed heterozygosity; H_E = NEI (1978)'s unbiased gene diversity; F_{IS} = WEIR and COCKERHAM (1984)'s estimate; TD = touchdown PCR.

* Indicates significant deviation from HWE at nominal level (α = 0.05); ** significant deviation from HWE after Bonferroni correction.

† Indicates markers with outlying F_{IS} (**Figure 2-1b**) and evidence of genotyping errors, thus, excluded from calculation of loci means and LD analyses.

‡ Product sizes include 18 bp addition of M-13 primer sequence. Different weights of florescent dyes influenced the product size by 1-2 bp.

§ Micro-Checker (VAN OOSTERHOUT *et al.* 2004) was used to detect the possible presence of genotyping error under HWE assumption.

Table 2-4 Marker density and intervals (cM) of the white-tailed deer and mule deer microsatellite marker panels.

Species		LG 23	LG 11	LG 5	Total
White-tailed deer	Number of markers	17	18	25	60
	Mean interval (± SD) cM	5.61 (± 3.29)	5.79 (± 6.54)	7.03 (± 5.48)	6.26 (± 5.28)
Mule deer	Number of markers	15	13	14	42
	Mean interval (± SD) cM	6.56 (± 3.93)	8.00 (± 7.98)	14.81 (± 9.51)	9.75 (± 8.11)

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CHAPTER 3 - Association Studies of Genetic Risk Factors for Chronic Wasting Disease in White-tailed Deer and Mule Deer

3.1 Introduction

Susceptibility to transmissible spongiform encephalopathies (TSEs), including chronic wasting disease (CWD) in deer, is most certainly underlain by a number of genetic and environmental risk factors. Uncovering genetic risk factors can be a useful approach for understanding pathogenic mechanisms and potentially identifying prophylactic and therapeutic targets. In TSEs, or prion diseases, a misfolded isoform (PrP^{Sc}) of the host-expressed prion protein (PrP^{c}) constitutes the unit of infection and propagation (PRUSINER 1989). Polymorphisms in the prion protein gene (*PRNP*) coding region were thus first targeted and shown to have major effects on human TSEs (reviewed in AGUZZI 2006; MEAD *et al.* 2009) and scrapie susceptibility (reviewed in GOLDMANN *et al.* 2005). In cattle, polymorphisms not in the coding region but in a promoter region were found to influence *PRNP* expression levels and BSE susceptibility (SANDER *et al.* 2004; SANDER *et al.* 2005; KASHKEVICH *et al.* 2007). *PRNP* alleles were also found to be disproportionately distributed among CWD infected and non-infected deer and elk in natural populations (O'ROURKE *et al.* 1999; JOHNSON *et al.* 2003; O'ROURKE *et al.* 2004; JEWELL *et al.* 2005; JOHNSON *et al.* 2006; WILSON *et al.* 2009) and significantly affected the speed of disease progression in experimental infection (HAMIR *et al.* 2006; O'ROURKE *et al.* 2007). *PRNP*, however, is clearly not the only risk factor as many quantitative trait loci (QTL) were mapped to other regions of the mouse (STEPHENSON *et al.* 2000; LLOYD *et al.* 2001; MANOLAKOU *et al.* 2001; LLOYD *et al.* 2002; MORENO *et al.* 2003), sheep (MORENO *et al.* 2008) and cattle genomes (HERNANDEZ-SANCHEZ *et al.* 2002; ZHANG *et al.* 2004), suggesting the presence of other important risk genes.

Over the past decade, advances in high-throughput sequencing and genotyping technology have revolutionized methods of detecting genetic risk factors for complex diseases and enabled linkage disequilibrium (LD) based association mapping, or whole-genome association studies (KRUGLYAK 1999; HIRSCHHORN and DALY 2005; MCCARTHY *et al.* 2008). While issues with study design and interpretation still remain (WANG *et al.* 2005; MCCARTHY *et al.* 2008), this approach allows fine-scale mapping

with genome-wide coverage and improved efficiency over the traditional candidate gene and QTL mapping approaches (HIRSCHHORN and DALY 2005). Benefitting from the human and cattle genomic resources already in place, the whole genome association studies of human TSEs and BSE have recently revealed highly localized genomic regions associated with TSE susceptibility (MEAD *et al.* 2009; MURDOCH *et al.* 2010). To conduct association mapping for CWD, however, the required genomic resource is still lacking. I previously assessed the utility of the high density bovine microsatellite map (IHARA *et al.* 2004) by evaluating the efficiency of cross-species amplification and extent of LD in CWD-affected deer populations (**Chapter 2**). However, this comparative approach only provided sparse marker density.

In this chapter I tested for CWD risk association using the marker panels developed in **Chapter 2** because the three linkage groups I targeted (LGs: red deer LG23, 11 and 5) were selected based on the predicted assignment of TSE candidate genes and QTLs. There were three candidate gene regions: *PRNP* on LG23, *IL1B* and *1L1RN* on LG11, and *NF1* on LG5, each of which were tagged with at least one nearby marker (< 1 cM). *IL1B* and *1L1RN* are members of interleukin-1 (IL-1) family coding IL-1β and its receptor antagonist IL-1RA, respectively. They are mediators in inflammatory response and risk factors for Alzheimer's disease (SCIACCA *et al.* 2003; LICASTRO *et al.* 2004), with additional evidence for functional and positional (QTL) links to TSE (SCHULTZ *et al.* 2004; MARCOS-CARCAVILLA *et al.* 2007). Neurofibromin 1 gene (*NF1*) is considered a tumor suppressor gene and responsible for inherited neurofibromatosis type 1 disorder, symptoms of which include neurofibroma, café-au-lait spots, Lisch nodules, and learning impairments (TROVÓ-MARQUI and TAJARA 2006). Although no direct connection has been suggested, the *NF1* region is a strong positional candidate for TSE (e.g. STEPHENSON *et al.* 2000; LLOYD *et al.* 2001; LLOYD *et al.* 2002; GELDERMANN *et al.* 2006). The three LGs also harbored additional predicted QTL outside the candidate regions (ZHANG *et al.* 2004). My marker panels, thus, offered a platform to conduct LD-based association tests for the target regions, while using a large number of intermittently spaced markers to test baseline associations (i.e. confounding) and other potential risk loci.

Association studies in emerging infectious diseases are often limited by the availability of naturally infected samples. The key to achieving an efficient study design, such as matched case-control (KLEINBAUM *et al.* 1982), is knowledge of various

epidemiological parameters, including spatial patterns in disease prevalence, modes of transmission, and demographic and environmental risk factors. This is because factors affecting an individuals' probability of infection can confound association tests. In CWD, key environmental risk factors (i.e. spatial location, age, and sex) have been characterized in major disease foci as a result of extensive surveillance programs (MILLER and CONNER 2005; GREAR *et al.* 2006; JOLY *et al.* 2006; OSNAS *et al.* 2009). More difficult to entangle have been the transmission patterns, but horizontal transmission was discovered to be the major source of CWD spread (MILLER and WILLIAMS 2003), suggesting social behavior and movement of deer should play a key role in defining transmission patterns (MILLER and WILLIAMS 2003; GREAR *et al.* 2010; CULLINGHAM *et al.* 2011). In conducting the genetic association tests, I employed the matched case-control study design to control for as many known confounding effects as possible.

3.2 Materials and Methods

3.2.1 Matched Case-control Samples

I used the same study populations as in the previous chapter, white-tailed deer (*Odocoileus virginianus*) from Wisconsin and mule deer (*O. hemionus*) from Saskatchewan. Study areas, sample collection, and CWD testing procedures are described in **Chapter 2** (**Section 2.2.2**). I individually matched CWD case and control samples according to known confounding risk factors. Firstly, I matched them by spatial location as CWD prevalence varies significantly across landscape as well as locally (MILLER and CONNER 2005; JOLY *et al.* 2006; OSNAS *et al.* 2009). Secondly, I matched samples by sex as both white-tailed deer and mule deer males have higher infection rates than females, supposedly due to their larger home range and more dynamic social interactions compared to females that are philopatric and have more closed social association (MILLER and CONNER 2005; GREAR *et al.* 2006; OSNAS *et al.* 2009). Thirdly, I matched samples by age as fawns and yearlings are known to have significantly reduced probability of infection compared to adults (MILLER and CONNER 2005; GREAR *et al.* 2006; OSNAS *et al.* 2009). Specific criteria for each species varied and are described below.

3.2.1.1 White-tailed Deer Samples

I obtained 96 pairs (*N*=192) of matched case-control samples of white-tailed deer from Wisconsin. To control for the broad-scale spatial heterogeneity in disease prevalence, I selected samples from within the CWD core zone, the central epidemic area (~303 km^2) in southwestern Wisconsin where the prevalence is the highest (6.2 % in 2002 –2004; JOLY *et al.* 2006). The core zone is geographically more confined than the CWD management zone (formerly, the herd reduction zone), from which I previously obtained the CWD negative samples (refer to Figure 1 in GREAR *et al.* 2006). Matching criteria were as follows: 1) only female deer were used; 2) only adults ≥ 2 years old were included; 3) case-controls were matched not only by the age at death but also by the year of kill (between year 2003 and 2009) whenever possible to minimize any potential temporal effects of the disease spread; 4) for each case sample I selected a control sample collected from the same one square mile section (~2.6 km^2), and if none was available, a match was selected randomly from one of the immediately adjacent sections.

3.2.1.2 Mule Deer Samples

The genotype data of the Saskatchewan mule deer samples collected in **Chapter 2** were used to test disease association (see **Section 2.2.2.2**). I excluded a small number of samples from the northern CWD range along the North Saskatchewan River as they belonged to a separate disease focus from the majority of samples collected in the South Saskatchewan River Valley (see Figure 1 in WILSON *et al.* 2009). Thus, the matched sample consisted of 87 pairs (*N* = 174), 47 male pairs and 40 female pairs all at the age of ≥1.5 years old. For each case sample, a control with the same or similar age and closest geographic location was selected. Due to limited sample availability, mean (± standard deviation) distance between pairs was 14.9 (± 12.1) km, ranging from 1.9 to 57.2 km. Also, sampling years could not be matched because the case samples were from throughout 2002 to 2006 while the controls were from 2005 and 2007 only.

3.2.2 DNA Extraction

I extracted DNA from the matched white-tailed deer samples using the same phenol-chloroform extraction protocol described in **Section 2.2.3.1** at the Department of Wildlife Ecology at the University of Wisconsin–Madison. Preserved tissues for this set of samples were lymph nodes, which were stored frozen. The extraction procedure of the matched mule deer samples is described in **Section 2.2.3.2**.

3.2.3 Marker Panels

The marker panels were previously developed from the bovine microsatellite map (IHARA *et al.* 2004) for three predicted deer LGs (LG23, 11, and 5). They were selected based on the chromosomal conservation with cattle and the predicted assignment of TSE candidate genes and QTL (see **Section 2.2.1**; **Table 2-1**). Through the cross-species amplification of the bovine markers, I previously obtained panels of 60 markers in white-tailed deer (**Figure 2-2**) and 42 markers in mule deer (**Figure 2-3**) (for marker information see **Table 2-2**; **Table 2-3**).

3.2.4 Genotyping

I genotyped the white-tailed deer matched samples using the panel of 60 markers. The same protocols for PCR, fragment analysis, and genotyping as described in the previous chapter were followed (**Section 2.2.4** and **2.2.6**). Due to recalibration of thermocyclers, I re-optimized PCR conditions for each marker by adjusting the annealing temperature and the concentration of Mg^{2+} (see **Table 3-1** for the new conditions). Failed samples were re-ran to obtain complete (> 95%) genotypes.

3.2.5 Diversity Statistics

Number of alleles, observed (H_O) and expected heterozygosity (H_E), and F_{IS} were calculated for each locus and averaged over all loci (outliers excluded) in the Excel Microsatellite Toolkit (PARK 2001) (for details see **Section 2.2.7**). Markers with outlying F_{IS} were identified using a natural break in F_{IS} distribution (plotted in SigmaPlot v11.0; Systat Software Inc, San Jose, CA, USA), which corresponded to roughly one standard deviation from the mean. These outliers were excluded from the following analyses. I also performed tests of Hardy-Weinberg equilibrium (HWE) in Genepop v4.0 (ROUSSET 2008). Pairwise testing of genotypic LD was also conducted in Genepop v4.0 by pooling rare alleles of frequency < 0.05 (for details see **Section 2.2.11.2**).

3.2.6 Association Testing

I conducted conditional logistic regression analysis (KLEINBAUM *et al.* 1982) to test CWD association in the matched data using in SAS v9.2 (SAS Institute Inc., Cary, NC, USA). This method takes into account the study design by treating matched pairs as

separate strata (KLEINBAUM *et al.* 1982). For each locus, the probability of infection was modeled using presence/absence of each allele as exposure variables. Rare alleles (< 0.05) were again pooled to maintain statistical power. The significance of the models was determined by likelihood ratio tests. Resulting *P*-values (negative log transformed) were plotted against predicted marker positions inferred from the bovine map (IHARA *et al.* 2004) using MapChart v2.1 (VOORRIPS 2002) to illustrate the occurrence of significant associations relative to predicted candidate gene and QTL locations. Bonferroni correction was used to obtain LG-wide and study-wide significance levels.

3.3 Results

3.3.1 Genotyping and Diversity Statistics

Four markers (DIK4520 on LG23; VH98, BM9138 and X82261 on LG5) failed to amplify complete sample genotypes (> 95%) in white-tailed deer and thus were excluded from the analysis. **Table 3-1** shows diversity statistics for each of the 56 amplified loci, its optimal PCR condition, and potential genotyping errors. In the white-tailed deer sample I observed 9.8 ± 5.0 (mean ± standard deviation) alleles per locus, H_O of 0.69 (± 0.23), H_E of 0.70 (± 0.23) and F_{IS} of 0.019 (± 0.035). While the mean number of alleles, H_O and H_E were highly similar to the CWD-negative white-tailed deer sample genotyped in the previous chapter (**Table 2-2**), the mean F_{IS} was substantially lower in the matched sample.

I identified three additional outlying loci (UMBTL184 and BM3501 from LG11; DIK2200 from LG5) in the F_{IS} distribution that had to be excluded from the following analysis due to abnormally high F_{IS} (> 0.1) (**Figure 3-1**; **Table 3-1**). All three loci were in significant deviation from HWE (**Table 3-1**). For the genotyping results of mule deer, see **Figure 2-1b** and **Table 2-3**.

3.3.2 LD in Candidate Regions

In the *PRNP* region on LG23, the BMS1669_URB021B pair, at a predicted distance of 0 cM on the bovine map (**Figure 3-2a,b**), was in strong LD in white-tailed deer ($p < 0.001$) and weakly linked in mule deer ($p = 0.08$). In the *IL1B* and *IL1RN* region, the UMBTL184_BM6445 pair, again at a predicted distance of 0 cM (**Figure**

3-3b), was in strong LD in mule deer ($p < 0.001$). UMBTL184 was not tested in white-tailed deer due to suspected genotyping error (**Table 3-1**), and thus, LD estimate in this region was not obtained in this species. In the *NF1* region, the DIK4009_DIK5136 pair, predicted to be within 0.6 cM (**Figure 3-4a,b**), was in strong LD in both white-tailed deer ($p < 0.001$) and mule deer ($p < 0.01$).

3.3.3 Association Testing

Of the 53 and 42 markers tested in white-tailed deer and mule deer, respectively, none were significantly associated with CWD status at LG-wide ($\alpha = 0.002$ to 0.004) or the study-wide significance levels ($\alpha = 0.0009$ to 0.001) (**Table 3-1**). Only two markers in total showed significant association at the nominal level ($\alpha = 0.05$) (**Table 3-1**): BMS1669 ($p = 0.006$) on LG23 in white-tailed deer (**Figure 3-2a**) and DIK5136 ($p = 0.02$) on LG5 in mule deer (**Figure 3-4b**). Both of these markers were predicted to be near (< 1 cM) the candidate genes, BMS1669 near *PRNP* and DIK5136 near *NF1*, and each of them was in strong LD with another nearby marker ($\leq$ 0.6 cM) in the same region (see **Section 3.3.2**). They were also nearly significant in the other species: BMS1669 in mule deer at $p = 0.05$ (**Figure 3-2b**) and DIK5136 in white-tailed deer at $p = 0.08$ (**Figure 3-4a**). Markers located near *IL1B* and *IL1RN* were non-significant in both species (**Figure 3-3a,b**); although one of them (UMBTL184) was nearly significant in mule deer ($p = 0.09$), this marker was not on the white-tailed deer panel due to high F_{IS}. Other markers supposedly located within previously identified QTL regions were non-associated. There were, however, several markers that showed nearly significant associations (**Figure 3-2**; **Figure 3-3**; **Figure 3-4**), while none did so consistently in both species. The detailed results of the association tests for each marker are listed in **Appendix II** and **Appendix III**.

3.4 Discussion

Past research on genetic risk factors for CWD has focused on coding polymorphisms within *PRNP* (e.g. O'ROURKE *et al.* 1999; JEWELL *et al.* 2005; JOHNSON *et al.* 2006; WILSON *et al.* 2009). Apart from *PRNP*, the only other candidate gene characterized so far has been the complement component C1q investigated by BLANCHONG *et al.* (2009). Though molecular evidence suggests its involvement in PrP^{Sc} facilitation during early stages of infection (KLEIN *et al.* 2001; MITCHELL *et al.* 2007),

polymorphism in C1q was found not to be associated with CWD in white-tailed deer (BLANCHONG *et al.* 2009). In the present study I indirectly interrogated *PRNP* and two new candidate regions with notable functional and/or positional significance, namely *IL1B/1L1RN* and *NF1*. Previously I identified weak LD (< 1 cM) in my target populations of CWD-affected white-tailed deer and mule deer (**Chapter 2**). This allowed fine-scale mapping in the candidate regions where I had sets of tightly linked markers (**Section 3.3.2**) that were also expected to be in LD with nearby candidate sites. While my marker panels only intermittently covered the rest of the LGs, the numerous, supposedly neutral markers provided an opportunity to identify confounding effects in my samples while also scanning for other risk loci.

Of all the association tests conducted, only two in total, BMS1669 in white-tailed deer (**Figure 3-2a**) and DIK5136 in mule deer (**Figure 3-4b**), showed suggestive association with CWD at the nominal level. More importantly both these markers were predicted to reside in two of the three candidate gene regions; *PRNP* and *NF1* regions, respectively. That the same markers also showed nearly significant association ($p = 0.05$ and $p = 0.08$, respectively) in the other species (**Figure 3-2b**; **Figure 3-4a**) provides further support for the associations being genuine. Furthermore, because *PRNP* is a known risk factor for CWD (e.g. JOHNSON *et al.* 2006; WILSON *et al.* 2009), the result highlights the ability to indirectly detect the disease association through linked markers even under the weak population LD. The fact that I did not observe an excess number of significant associations at the unlinked loci (**Table 3-1**) supports the idea that spurious association due to genetic substructure should be minimal in my samples (**Chapter 2 Section 2.4.2**). On the flip side, my overall results exemplified the difficulty in detecting highly significant association at LG-wide or study-wide levels. While the matched case-control design is an efficient study design (KLEINBAUM *et al.* 1982), my sample sizes were likely still small relative to the size of the marker panels. Also the use of small sample size and low marker density likely undermined my ability to detect risk alleles, especially those with low penetrance, or only modest effects on the disease susceptibility. Another statistical challenge was the high genetic diversity found in my populations, which often resulted in reduced power from the large numbers of microsatellite alleles (**Table 3-1**).

The detection of the significant association near *PRNP* corroborates the mounting evidence for the *PRNP* polymorphism as an important risk factor for CWD. Across

species and regions, however, the consensus appears that associated alleles predispose animals to differences in incubation periods but not complete resistance (HAMIR *et al.* 2006; O'ROURKE *et al.* 2007), and they do not confer a molecular barrier in wild populations (O'ROURKE *et al.* 1999; JOHNSON *et al.* 2003; O'ROURKE *et al.* 2004; JEWELL *et al.* 2005; JOHNSON *et al.* 2006; WILSON *et al.* 2009). In Wisconsin white-tailed deer, certain allelic combinations at codon 95 and 96 were found to be overrepresented in non-infected animals, suggesting a relative resistance effect. However, these genotypes were rare, and combinations of alleles associated with relative susceptibility were found in over 90 % of the population (JOHNSON *et al.* 2003; JOHNSON *et al.* 2006). Similarly in Saskatchewan mule deer, polymorphisms at codon 20 was found to be disproportionately distributed between infected and non-infected animals, but genotypes associated with relative resistance were also predominant among infected animals, indicating a weak effect (WILSON *et al.* 2009). My association results at BMS1669 (~0.6 Mb from *PRNP*; **Figure 3-2**), thus, most likely reflect these partial effects of *PRNP* alleles in the population. However, in some cervid populations the coding polymorphism is known to be unrelated to CWD susceptibility (PERUCCHINI *et al.* 2008). Therefore, it remains a possibility that other causative polymorphisms outside the *PRNP* coding regions affect expression of *PRNP* or function/expression of other linked genes. The fact that the other non-significant tag marker (URB021B) (**Figure 3-2**) is located much farther from *PRNP* (~1.4 Mb) in the bovine reference genome (NC_007311.4; ELSIK *et al.* 2009) still supports that the risk gene is closer to *PRNP*.

The detection of the associations in the *PRNP* region also has an important implication for the adequacy of the matched case-control samples. So far the known genetic risk factor (i.e. *PRNP* genotypes) affects only the incubation periods and disease progression, and all animals are thought to eventually succumb to CWD (HAMIR *et al.* 2006; O'ROURKE *et al.* 2007). This would mean that the use of controls in wild populations rests on a precarious assumption that the absence of detectable PrP^{Sc} accumulation in tested tissues indicates slower disease progression, while it may well be a consequence of lower exposure levels. While I attempted to minimize the exposure effect by spatially matching the case-control samples, actual exposure in the wild cannot be measured, and potential inclusion of many inadequate controls could conceal genetic associations with relative susceptibility. In spite of this uncertainty, the marker in the *PRNP* region, the known genetic risk factor, served as a positive control for my samples.

Given that the marker alleles are in LD with *PRNP* alleles, the significant and nearly significant associations found in the *PRNP* region in the white-tailed deer and mule deer, respectively (**Figure 3-2**), suggest the provisional effectiveness of the control samples and thereby the validity of the association results for the marker panels. Therefore, it will be desirable to follow-up by obtaining the *PRNP* genotypes and confirming the LD phases between the marker and the *PRNP* loci. In a future study, diagnosing and scoring the different stages of PrP^{Sc} accumulation (e.g. JOHNSON *et al.* 2006) might also help more closely associate genetic risk factors with disease progression.

In addition to *PRNP*, experimental QTL studies have identified many other genetic risk factors but often suffered inconsistent results due to the use of different lines of inbred mice and prion strains (e.g. STEPHENSON *et al.* 2000; LLOYD *et al.* 2001; MANOLAKOU *et al.* 2001). The *NF1* region, however, is one that has been implicated by multiple studies. It is contained within QTL on mouse chromosome 11 mapped for experimental scrapie (STEPHENSON *et al.* 2000; LLOYD *et al.* 2001) and BSE incubation periods (LLOYD *et al.* 2002). ZHANG *et al.* (2004) also detected a suggestive QTL harbouring this region on cattle chromosome 19 in naturally BSE-infected cattle. Furthermore, GELDERMANN *et al.* (2006) targeted the *NF1* region and found significant associations at two surrounding markers (~4 cM apart in IHARA *et al.* 2004) in various case-control BSE breeds. Thus, the association I found in mule deer (**Figure 3-4b**), the first evidence for an association with CWD at this region, supports the idea that the *NF1* region likely contains risk loci. While it is critical to note that a number of other studies did not detect QTL or associations in this region (MANOLAKOU *et al.* 2001; HERNANDEZ-SANCHEZ *et al.* 2002; MORENO *et al.* 2003; MURDOCH *et al.* 2010), the usual difficulty of replicating QTL and association results and the fact that the *PRNP* region was not always found significant by the previous mapping studies (e.g. HERNANDEZ-SANCHEZ *et al.* 2002; ZHANG *et al.* 2004; MURDOCH *et al.* 2010) suggests that the *NF1* region merits further investigation.

The ascertainment of the actual risk genes from previously identified QTL regions is hampered by the vast number of genes harboured within a QTL region (HIRSCHHORN and DALY 2005). With the limited LD (< 1 cM) in white-tailed and mule deer I may be able to narrow down candidates in the *NF1* region. Based on the bovine reference genome sequence (NC_007317.4; ELSIK *et al.* 2009), the other non-significant tag marker (DIK4009) is located closer (~0.8 Mb) to the *NF1* locus than DIK5136 (~1.6

Mb), while the markers themselves were in significant LD in both deer species. This may suggest that the actual risk gene is located closer to DIK5136 and not *NF1*. While no clear connection between *NF1* and TSEs has been suggested so far, the region surrounding (~0.5Mb) DIK5136 has a high density of genes including some that are potentially relevant to TSE (e.g. genes encoding: microRNAs, *MIR451* and *MIR144*; a lipid raft protein flotillin-2; a glycolytic enzyme aldolase-C). For example, microRNAs (miRNAs) regulate gene expression via RNA silencing, and dysfunctional regulation by some miRNAs was recently implicated in accumulation of amyloid proteins in Alzheimer's disease and TSEs (reviewed in PROVOST 2010). Also, alteration in lipid raft constituents have been hypothesized to play a role in a variety of neurodegenerative diseases including TSEs (reviewed in SCHENGRUND 2010). PrP^c is a glycosyl-phosphatidylinositol (GPI) anchored protein associating with lipid rafts and suspected to interact with fotillin-2 and flotillin-1 in various signaling pathways (SCHENGRUND 2010; SOLIS *et al.* 2010). Similarly, aldolase-C was identified as an interactor of PrP^c (STROM *et al.* 2006), and its long transcripts were found to be overexpressed in BSE-infected mice (DANDOY-DRON *et al.* 2000). Thus, targeted investigation of these and other genes close to DIK5136 may prove to be an effective starting point for mining the *NF1* region.

The failure to detect significant association near *IL1B* and *IL1RN* (**Figure 3-3**) suggests that these genes unlikely contribute strong risk, especially knowing that the tag markers were predicted to be within 0.1 – 0.2 Mb (NC_007309.4; ELSIK *et al.* 2009), well in the expected range of strong LD in these populations. It should be noted, however, that one of the markers (UMBTL184) was nearly significant ($p = 0.09$) in mule deer (**Figure 3-3b**) and was not tested in white-tailed deer due to high probability of null alleles (**Table 3-1**). Thus, the lack of association in the *IL1B* and *IL1RN* region may need to be examined further. Other evidence linking these genes to TSEs appears substantial. Functionally, IL-1 members, pro-inflammatory cytokines, are hypothesized to promote neuronal damage in TSE and Alzheimer's disease by inducing cytotoxicity (BURWINKEL *et al.* 2004). This was demonstrated by upregulation of IL-1β and its receptor IL-1RI in scrapie-infected mice as well as resistance of IL-1RI knockout mice to scrapie (SCHULTZ *et al.* 2004). Furthermore, MARCOS-CARCAVILLA *et al.* (2007) detected overexpression of both *IL1B* and *IL1RN* in the cerebellum of scrapie-infected sheep. Positionally, QTL intervals mapped to mice chromosome 2 (LLOYD *et al.* 2001; MANOLAKOU *et al.* 2001; LLOYD *et al.* 2002) were found to harbor *IL1B/IL1RN* loci and correspond to locations of

these genes identified on sheep chromosome 3 (MARCOS-CARCAVILLA *et al.* 2007). The QTL intervals are, however, very large and contain a substantial number of other genes; thus, thorough investigation of the surrounding region would be required to determine whether or not the IL-1 genes actually contribute to TSE risk and whether there are other risk genes.

Finally, the marker density was clearly insufficient for the rest of the predicted QTL regions assigned to my LGs (**Figure 3-2**; **Figure 3-3**; **Figure 3-4**); thus, the lack of association in my marker panel by no means indicates absence of other risk genes. However, because of the weak LD and the marker deficiency in deer, thorough investigation of these large untargeted portions of LGs would have to wait until the development of genomic resources (i.e. high density SNP panels).

3.5 Conclusion

In the present study, I used the matched case-control design to control for confounding factors in CWD prevalence, and using the bovine-sourced marker panels, identified CWD associations near *PRNP* and *NF1*. The identification of a new candidate region for CWD is particularly significant because it is consistent with evidence from other TSEs in both experimental and natural studies, and thereby implies a common process underlying susceptibility. Therefore, further investigation of the *NF1* region may aid in uncovering important risk genes and susceptibility mechanisms.

A few areas of uncertainty accompanying my results, however, need to be addressed. The first is the use of bovine primers and potential errors in amplification and genotyping. I previously showed that the cross-amplification rate was low (< 30%), indicating significant sequence divergence between cattle and white-tailed deer (**Chapter 2 Section 2.4.1**). This issue was also evident in the high sensitivity of many primer sets to PCR conditions. The second issue is also associated with the comparative method and is the lack of loci position information in deer. While large scale deer-cattle genome conservation was previously confirmed in red deer (SLATE *et al.* 2002), I had to infer fine-scale loci positions where the extent of conservation has not yet been characterized. Finally, confounding effects may still be a problem, especially in mule deer where spatial matching was probably inadequate. Although cryptic population substructure was determined to be negligible (**Chapter 2 Section 2.4.2**), both white-tailed deer and mule

deer females from these areas tightly associate with kin and likely share elevated risk of CWD infection (GREAR *et al.* 2010; CULLINGHAM *et al.* 2011a; CULLINGHAM *et al.* 2011b). Thus, significant sampling bias toward such related females is a potential concern. While I did not find excess association at supposedly neutral markers in the same LGs, better matching and increased sample sizes are desirable in the future.

Lastly, my study suggests that future work characterizing genes in the *NF1* candidate region in the deer genome via comparative approach and testing for CWD association is warranted. This should be followed by validation studies using independent samples from different CWD regions, perhaps using CWD-affected deer from Colorado and Wyoming where a large number of samples are available due to the long history and high prevalence of CWD (WILLIAMS 2005; SIGURDSON 2008). CWD-affected elk in the contiguous area may also be investigated to see whether similar association is found in the third species. Moreover, relatedness information could be incorporated into matching criteria as done by BLANCHONG *et al.*(2009). This method should be feasible where a large number of control samples are available from extensive surveillance efforts. Finally, with the emerging genomic resources for white-tailed deer (SEABURY *et al.* 2011) (MALEFANT *et al.* unpublished data), a physical map may become available in the near future for characterizing fine-scale LD and developing SNP-typing systems that can be used for whole-genome association studies. However, this approach usually requires substantial sample sizes (HIRSCHHORN and DALY 2005); thus, feasibility for CWD will need to be determined.

3.6 Figures and Tables

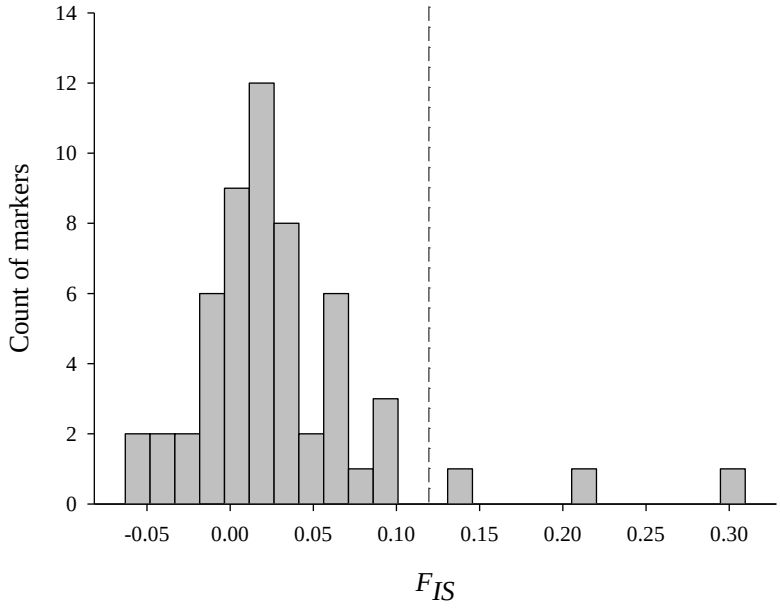

Figure 3-1 F_{IS} distribution of 56 markers in white-tailed deer (N = 192). Dashed line indicates natural break (roughly corresponding to one standard deviation from the mean) used to identify the outlying loci with high F_{IS} , which were excluded from the subsequent analyses.

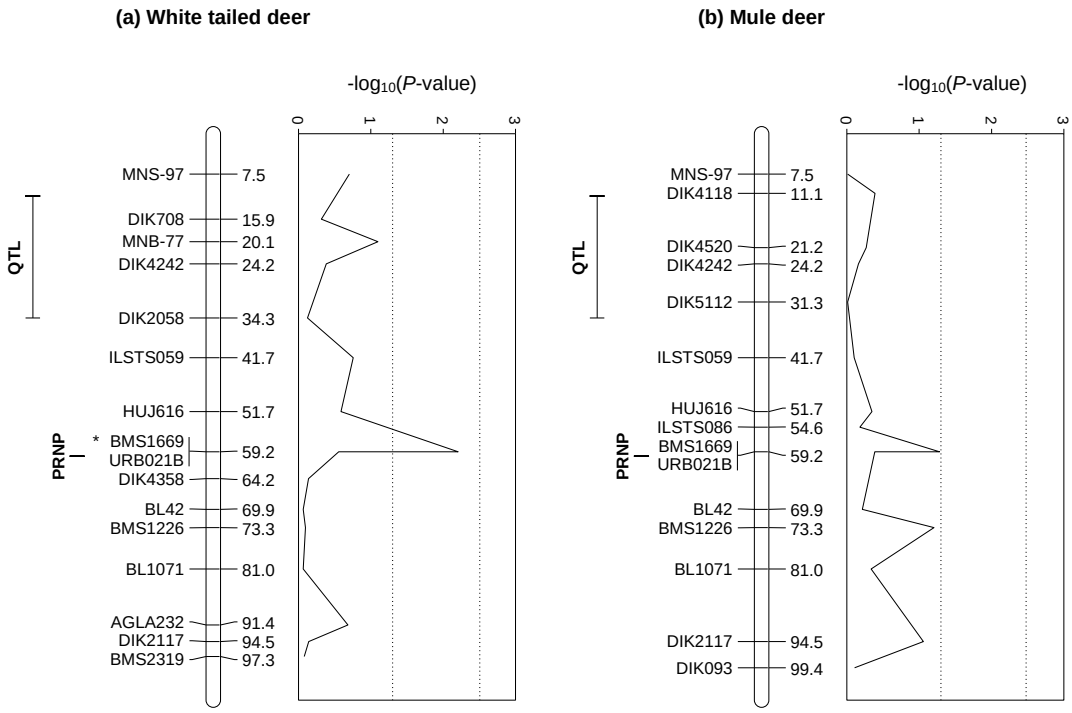

Figure 3-2 Predicted positions of the microsatellite markers, candidate genes, and QTL on red deer LG23 (Bta13) and the probability of marker associations with CWD in the matched case-control samples of white-tailed deer (a) and mule deer (b). Vertical lines show nominal and LG-wide significance levels, respectively. Markers above the nominal significance are indicated with *. Marker positions were inferred from the high density bovine microsatellite map (IHARA *et al.* 2004).

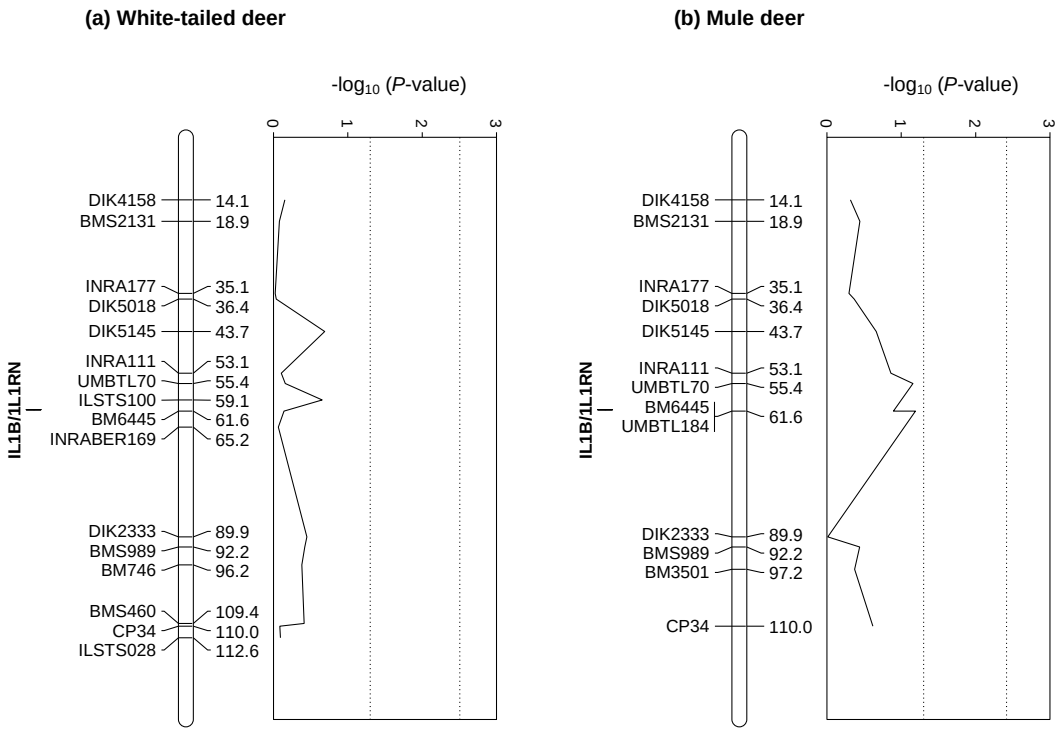

Figure 3-3 Predicted positions of the microsatellite markers, candidate genes, and QTL on red deer LG11 (Bta11) and the probability of marker associations with CWD in the matched case-control samples of white-tailed deer (a) and mule deer (b). Vertical lines show nominal and LG-wide significance levels, respectively. Markers above the nominal significance are indicated with *. Marker positions were inferred from the high density bovine microsatellite map (IHARA *et al.* 2004).

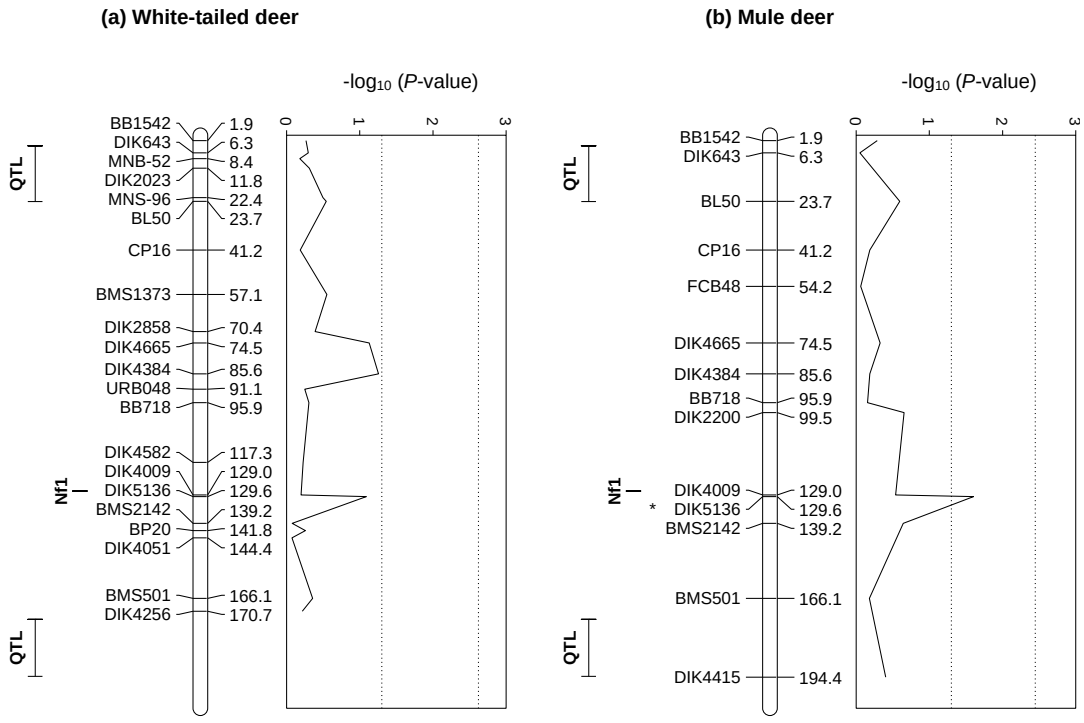

Figure 3-4 Predicted positions of the microsatellite markers, candidate genes, and QTL on red deer LG5 (Bta17 & 19) and the probability of marker associations with CWD in the matched case-control samples of white-tailed deer (a) and mule deer (b). Vertical lines show nominal and LG-wide significance levels, respectively. Markers above the nominal significance are indicated with *. Marker positions were inferred from the high density bovine microsatellite map (IHARA *et al.* 2004).

Table 3-1 PCR conditions and diversity statistics for the microsatellite marker panel in the white-tailed deer matched sample and the results of CWD association tests in white-tailed deer (*N*=192) and mule deer (*N*=174).

Marker	Ta	Mg2+ (mM)	M13	Number of alleles	Size range (bp) ‡	H_O	H_E	F_{IS}	White-tailed deer		Mule deer	
									χ^2(df)	*P*	χ^2(df)	*P*
AGLA232	48 °C	2	PET	2	164-165	0.391	0.416	0.062	3.15(2)	0.21	-	-
BB1542	46 °C	2	FAM	9	266-296	0.686	0.736	0.068	5.00(6)	0.54	5.22(6)	0.52
BB718	50 °C TD	3	FAM	11	152-176	0.862	0.864	0.003	7.38(8)	0.50	4.69(7)	0.70
BL1071	52 °C TD	2	VIC	17	181-213	0.777	0.804	0.034	3.23(7)	0.86	1.55(2)	0.46
BL42	46 °C	3	VIC	9	252-268	0.817	0.817	0.001	3.26(7)	0.86	1.83(3)	0.61
BL50	46 °C	3	NED	10	227-247	0.822	0.829	0.008	7.38(6)	0.29	6.58(5)	0.25
BM3501 †	56 °C TD	2	VIC	13	168-200	0.696	0.813	0.144**	-	-	2.80(3)	0.42
BM6445	46 °C	2	FAM	12	129-153	0.865	0.855	-0.012*	5.31(8)	0.72	8.58(5)	0.13
BM746	52 °C TD	2	FAM	10	160-182	0.794	0.805	0.014	8.19(8)	0.42	-	-
BMS1226	50 °C	2	VIC	11	160-180	0.820	0.872	0.060	5.37(9)	0.80	8.95(4)	0.06
BMS1373	50 °C TD	3	VIC	4	115-120	0.516	0.571	0.097	5.06(4)	0.28	-	-
BMS1669	46 °C	2	FAM	13	109-141	0.843	0.851	0.010	**21.40(8)**	**0.01**	16.78(9)	0.05
BMS2131	44 °C	3	PET	13	226-254	0.757	0.768	0.016	3.51(7)	0.83	7.70(7)	0.36
BMS2142	50 °C TD	3	NED	19	124-157	0.837	0.848	0.014	2.78(6)	0.84	5.65(4)	0.23
BMS2319	52 °C	2	PET	10	112-142	0.708	0.715	0.009	2.78(6)	0.84	-	-
BMS460	50 °C	3	NED	17	126-162	0.891	0.909	0.020	8.48(8)	0.39	-	-
BMS501	52 °C	2	VIC	16	134-172	0.786	0.864	0.090	8.97(9)	0.44	4.11(6)	0.66
BMS989	48°C TD	3	VIC	11	105-129	0.869	0.858	-0.013	8.57(8)	0.38	5.46(5)	0.36
BP20	50 °C TD	3	NED	3	224-227	0.414	0.419	0.014	2.09(3)	0.55	-	-
CP16	46 °C	3	VIC	14	90-126	0.853	0.846	-0.009	4.20(6)	0.65	2.47(4)	0.65
CP34	52 °C	2	FAM	10	117-143	0.781	0.795	0.017	2.19(5)	0.82	5.49(4)	0.24
DIK093	-	-	-	-	-	-	-	-	-	-	3.23(6)	0.78

Table 3-1 Continued.

Marker	Ta	Mg2+ (mM)	M13	Number of alleles	Size range (bp) ‡	H_O	H_E	F_{IS}	White-tailed deer		Mule deer	
									χ^2(df)	*P*	χ_2(df)	*P*
DIK2023	52 °C	2	PET	11	167-195	0.870	0.874	0.005	8.47(9)	0.49	-	-
DIK2058	52 °C	2	FAM	4	168-174	0.654	0.678	0.034*	1.92(4)	0.75	-	-
DIK2117	54°C TD	3	FAM	13	218-241	0.793	0.807	0.018	4.49(7)	0.72	11.03(6)	0.09
DIK2200 †	56 °C TD	2	VIC	15	167-189	0.586	0.849	0.310**	-	-	1.50(1)	0.22
DIK2333	52 °C	2	PET	11	199-219	0.649	0.704	0.078	6.64(6)	0.36	0.88(5)	0.97
DIK2858	50 °C TD	3	FAM	4	206-212	0.524	0.575	0.089	2.89(3)	0.41	-	-
DIK4009	52 °C TD	3	NED	14	194-221	0.853	0.898	0.050	7.93(10)	0.64	3.76(3)	0.29
DIK4051	50 °C TD	3	FAM	10	223-241	0.811	0.837	0.032	4.15(8)	0.84	-	-
DIK4118	-	-	-	-	-	-	-	-	-	-	6.13(6)	0.41
DIK4158	52°C	3	PET	16	237-275	0.911	0.893	-0.020	5.49(8)	0.70	1.52(5)	0.91
DIK4242	48°C TD	3	FAM	17	267-305	0.862	0.855	-0.009	7.14(7)	0.41	5.63(8)	0.69
DIK4256	52 °C	2	VIC	10	234-260	0.896	0.843	-0.063	5.40(7)	0.61	-	-
DIK4358	46 °C	2	NED	17	132-168	0.848	0.876	0.032	5.26(8)	0.73	-	-
DIK4384	50 °C	2	NED	13	222-246	0.801	0.859	0.068	16.60(9)	0.06	5.08(7)	0.65
DIK4415	-	-	-	-	-	-	-	-	-	-	2.98(3)	0.39
DIK4520	-	-	-	-	-	-	-	-	-	-	5.03(6)	0.54
DIK4582	50 °C TD	2	NED	4	219-233	0.524	0.558	0.061	2.79(4)	0.59	-	-
DIK4665	46 °C	3	NED	12	200-230	0.843	0.838	-0.005	12.91(7)	0.07	4.56(5)	0.47
DIK5018	52 °C	2	FAM	6	218-228	0.740	0.754	0.020	1.98(6)	0.92	3.83(4)	0.43
DIK5112	-	-	-	-	-	-	-	-	-	-	0.49(4)	0.97
DIK5136	46°C	3	VIC	10	260-278	0.665	0.674	0.014	9.79(5)	0.08	**16.03(7)**	**0.02**
DIK5145	50 °C TD	2	FAM	5	182-194	0.660	0.682	0.033	4.58(3)	0.21	7.05(5)	0.22

Table 3-1 Continued.

Marker	Ta	Mg2+ (mM)	M13	Number of alleles	Size range (bp) ‡	H_O	H_E	F_{IS}	White-tailed deer		Mule deer	
									χ^2(df)	*P*	χ^2(df)	*P*
DIK643	46 °C	2	VIC	6	177-187	0.774	0.734	-0.055	4.32(5)	0.50	0.64(3)	0.89
DIK708	60 °C TD	2	FAM	2	208-209	0.089	0.094	0.061	1.45(2)	0.48	-	-
FCB48	-	-	-	-	-	-	-	-	-	-	0.29(2)	0.86
HUJ616	52 °C	2	PET	8	124-138	0.791	0.813	0.027	6.53(5)	0.26	4.74(5)	0.45
ILSTS028	52 °C	2	NED	3	149-153	0.089	0.085	-0.041	0.06(1)	0.81	-	-
ILSTS059	52 °C	2	PET	11	167-197	0.719	0.749	0.040	8.98(6)	0.18	1.03(3)	0.79
ILSTS086	-	-	-	-	-	-	-	-	-	-	5.89(8)	0.66
ILSTS100	54 °C TD	2	NED	2	204-206	0.089	0.085	-0.044	1.49(1)	0.22	-	-
INRA111	46 °C	2	FAM	13	121-145	0.807	0.802	-0.007	2.43(5)	0.79	9.70(6)	0.14
INRA177	46 °C	2	NED	12	92-122	0.863	0.889	0.029	3.29(9)	0.95	3.32(4)	0.51
INRABER169	52 °C	2	VIC	9	207-234	0.782	0.792	0.012	3.25(7)	0.86	-	-
MNB-52	52 °C	2	FAM	2	185-187	0.354	0.354	0.000	0.83(2)	0.66	-	-
MNB-77	54 °C TD	2	NED	3	216-222	0.208	0.210	0.008	6.76(3)	0.08	-	-
MNS-96	46 °C	2	PET	3	180-186	0.105	0.110	0.045*	2.28(2)	0.32	-	-
MNS-97	50°C TD	3	VIC	5	269-281	0.387	0.398	0.026	6.02(4)	0.20	0.05(2)	0.97
UMBTL184 †	50 °C TD	3	PET	17	274-303	0.667	0.845	0.211**	-	-	11.89(6)	0.06
UMBTL70	50 °C TD	2	PET	14	173-205	0.893	0.866	-0.032	4.73(7)	0.69	13.10(7)	0.07
URB021B	54 °C	2	NED	6	145-155	0.750	0.762	0.016	6.29(5)	0.28	3.98(4)	0.41
URB048	50 °C TD	2	PET	21	176-206	0.906	0.912	0.007	6.73(8)	0.57	-	-
Mean				10		0.690	0.704	0.019				
SD				5		0.234	0.234	0.035				

Table 3-1 Continued.

H_O = observed heterozygosity; H_E = expected heterozygosity under Hardy-Weinberg equilibrium (HWE); F_{IS} by Weir & Cockerham (1984)'s estimate; TD = touchdown PCR protocol.
Associations were tested using conditional logistic regression and likelihood ratio tests. Significant results (α = 0.05) are shown in bold.

*Significant deviation from HWE at nominal level (α=0.05); ** significant deviation from HWE after Bonferroni correction.

†Markers with outlying F_{IS} (**Figure 3-1**) and evidence of genotyping error, thus, excluded from subsequent analysis.

‡Product sizes incude 18 bp addition of M-13 primer sequence to the forward primer. Different weights of four florescent dyes influenced the product size by 1-2 bp.

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CHAPTER 4 - Conclusion

4.1 Synthesis

There has been a surge of technologies and applications in genomics research over the past years. Next-generation sequencing is now capable of producing millions of sequence reads in a single reaction and becoming increasingly inexpensive (MARDIS 2008; ROKAS and ABBOT 2009). As a result, concerted genome sequencing efforts have led to the characterization of many genomes for economically important species and generated high-throughput genotyping tools (e.g. HILLIER *et al.* 2004; LINDBLAD-TOH *et al.* 2005; ELSIK *et al.* 2009). Further decline in sequencing cost is expected to bring similar benefits to other species of ecological and evolutionary importance (ROKAS and ABBOT 2009; SLATE *et al.* 2010; SEEB *et al.* 2011). In fact, during the course of my short study, the bovine genome sequence and characterization of genome-wide SNP variation were published (ELSIK *et al.* 2009; GIBBS *et al.* 2009); furthermore, completion of the genome-wide SNP discovery and transcriptome sequencing of white-tailed deer (SEABURY *et al.* 2011)(MALEFANT *et al.* unpublished data) occurred just over the last year. At the moment, however, most wild species still lag behind in the genomic era. Even for white-tailed deer, SNP panels customized for association mapping are unlikely to materialize instantly. Thus, considering the urgency of chronic wasting disease (CWD) spread and its impending impacts, it is worthwhile to explore other alternatives for studying genetic risk factors in deer. In my thesis, I ascertained the feasibility of association mapping in deer via the comparative use of the high density bovine microsatellite genetic map.

Cross-species amplification of bovine microsatellite markers in white-tailed deer revealed a transfer rate of ~28 %, which translates to well over 1,000 markers which can be transferred from the bovine map consisting of >3,800 microsatellites (IHARA *et al.* 2004). Since the existing genetic map of red deer contains only 153 microsatellites (SLATE *et al.* 2002), my results highlights the potential to bring the marker density to a few-centimogran (cM) interval. This hypothetical map would probably be sufficient for pedigree-based quantitative trait loci (QTL) mapping or association mapping in populations with strong linkage disequilibrium (LD). The characterization of LD in my target deer populations, however, showed weak LD hardly extending over 1 cM, and I

was unable to determine the precise extent due to the lack of marker density. This level of LD is consistent with high dispersal ability and elevated genetic diversity in these populations (e.g. VAN DEN BUSSCHE *et al.* 2002; DEYOUNG *et al.* 2003; LATCH *et al.* 2009) but implies a need for much denser marker sets to conduct association mapping. Based on the cross-species amplification estimate I obtained, the comparative approach will not provide necessary tools to map CWD genetic risk factors in naturally infected deer populations.

Following the proof of principle experiment, I also interrogated a few functional and positional candidate regions for CWD risk factors. Despite the lack of marker coverage, CWD association tests in the target populations were found effective due to the absence of population substructure, the availability of samples for matching case-controls, and the weak LD which provided high resolution in the target genomic regions. The detection of association at a marker near the prion protein gene (*PRNP*), the known CWD risk factor, demonstrated the effectiveness of the marker-assisted association tests via LD. Another notable result was the association in additional candidate region around the neurofibromin 1 gene (*NF1*). This finding provisionally represents the only genetic association found for CWD so far other than the *PRNP* and corroborates previously mapped QTL intervals of scrapie and bovine spongiform encephalopathy (BSE) incubation periods (STEPHENSON *et al.* 2000; LLOYD *et al.* 2001; LLOYD *et al.* 2002; ZHANG *et al.* 2004; GELDERMANN *et al.* 2006). In summary, the overall findings of my study provided both methodological and specific insights to the research of CWD genetic risk factors. This can be considered a step forward, knowing the tactics in the past have almost exclusively been the interrogation of polymorphisms in a single gene (i.e. *PRNP*).

4.2 Future Prospects

Dense SNP panels are absolute requirements for association mapping in deer given the weak LD I observed. However, whole genome association studies will likely remain inaccessible until species-specific SNP panels can be developed. In contrast to the cross-species utility of microsatellite primers, the bovine and ovine SNP chips are expected to be uninformative in cervids based on their limited utility (~1-4 % polymorphic) shown for much evolutionary closer species within Bovidae (MILLER *et al.* 2011; PERTOLDI *et al.* 2010). This also implies divergent cervid lineages such as red deer (genus *Cervus*) and white-tailed deer (genus *Odocoileus*) (FERNANDEZ and VRBA 2005;

GILBERT *et al.* 2006) will require their own SNP panels. Fortunately though, genomic resources of related species can facilitate genomics in wild species by offering the frameworks of assembled genomes and functional annotation (SEABURY *et al.* 2011; SEEB *et al.* 2011). Thus, we could expect efficient sequencing and characterization of a deer genome through capitalizing on the next-generation sequencing technology and the established bovine resources. The recent discovery of over 10,000 SNPs via the next-generation sequencing of a reduced representation library (SEABURY *et al.* 2011) and a blood transcriptome of white-tailed deer (MALENFANT, *et al.* unpublished data) indicates sufficient scientific interest in advancing genomics for this species.

Whole-genome association studies for CWD will have to wait until appropriate genomic tools are in place. In the meantime, the search for CWD genetic risk factors can continue via the traditional QTL mapping and candidate gene approaches. QTL mapping in mice using CWD strains may provide useful information for positional candidates; in particular, the wealth of previous QTL studies conducted with other TSE strains (e.g. STEPHENSON *et al.* 2000; LLOYD *et al.* 2001; MANOLAKOU *et al.* 2001; LLOYD *et al.* 2002) may allow discerning regions of common risk and CWD-specific factors. The genetic map of deer could be improved by the cross-amplification of bovine microsatellites; however, QTL mapping in deer may not be practical for CWD because of the limited number of captive cases (MILLER and WILD 2004) and the long experimental duration (HAMIR *et al.* 2006; O'ROURKE *et al.* 2007). Finally, the present study illuminated the potential of the case-control based, marker-assisted association tests in mining candidate regions. Because of weak LD, this approach could further localize candidate regions given sufficient marker density, as exemplified by the *NF1* region in this study. Also, the traditional candidate gene approach can be significantly improved by the use of the annotated bovine genome and newly available white-tailed deer contigs and SNPs (SEABURY *et al.* 2011)(MALENFANT, *et al.* unpublished data) as sources for primer design. Given the vast array of potential prion protein (PrP^{C}) interacting partners identified (reviewed in AGUZZI *et al.* 2008), candidate gene association studies may help narrow the list down to those actually implicated in the pathogenesis.

Faced with the enormous complexity of the prion pathobiology, genetic studies of TSEs should continue to search broadly for risk factors in order to provide insights from a new angle. CWD's exceptional transmissibility in the environment makes it a vital element in understanding the full scope of the TSE pathogenesis; at the same time, it

makes developing effective control measures an urgent priority. Cervid genomic resources being generated through new sequencing technologies are expected to supply tools for whole-genome association studies in the future and may lead to an unprecedented advance in the CWD research.

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Appendix I. Amplification of 215 bovine microsatellite markers in the white-tailed deer screening panel.

Marker	Deer LG	Cattle LG	Bovine position (cM)	White-tailed deer amplification
DIK083	23	13	0.0	Failed
DIK2325	23	13	1.0	Failed
DIK2576	23	13	3.7	Failed
UWCA21	23	13	5.8	Failed
MNS-97	23	13	7.5	Polymorphic at standard
TGLA23	23	13	9.0	Monomorphic at standard
DIK4118	23	13	11.1	Polymorphic after optimization
DIK708	23	13	15.9	Polymorphic after optimization
TGLA6	23	13	18.1	Failed
AF2	23	13	19.4	Failed
MNB-54	23	13	20.1	Failed
MNB-77	23	13	20.1	Polymorphic at standard
MNS-75	23	13	20.7	Failed
DIK4520	23	13	21.2	Polymorphic after optimization
BMS1742	23	13	23.0	Failed
DIK4242	23	13	24.2	Polymorphic at standard
DIK4536	23	13	29.5	Failed
DIK5112	23	13	31.3	Polymorphic after optimization
DIK2058	23	13	34.3	Polymorphic at standard
DIK2039	23	13	36.5	Failed
DIK5201	23	13	38.3	Failed
BMS1231	23	13	39.6	Failed
ILSTS059	23	13	41.7	Polymorphic at standard
DIK4178	23	13	45.0	Failed
DIK4057	23	13	47.4	Failed
DIK1120	23	13	49.2	Failed
HUJ616	23	13	51.7	Polymorphic at standard
DIK2961	23	13	53.1	Failed
ILSTS086	23	13	54.6	Polymorphic at standard
DIK5268	23	13	57.7	Failed
DIK4467	23	13	58.3	Monomorphic after optimization
BMS1669	23	13	59.2	Polymorphic after optimization
URB021B	23	13	59.2	Polymorphic at standard
BM4509	23	13	60.0	Monomorphic at standard
DIK054	23	13	61.8	Failed
DIK4358	23	13	64.2	Polymorphic after optimization
DIK4065	23	13	66.6	Failed
BL42	23	13	69.9	Polymorphic at standard
BMS1676	23	13	71.1	Failed
BMS1226	23	13	73.3	Polymorphic after optimization
BMS1784	23	13	75.1	Failed
DIK4350	23	13	77.1	Failed
BL1071	23	13	81.0	Polymorphic at standard
INRA196	23	13	84.4	Failed
DIK4871	23	13	87.0	Failed
AGLA232	23	13	91.4	Polymorphic after optimization
DIK2117	23	13	94.5	Polymorphic at standard
BMS995	23	13	96.0	Failed
BMS2319	23	13	97.3	Polymorphic at standard
DIK093	23	13	99.4	Polymorphic at standard
DIK4468	23	13	102.1	Failed
DIK2504	23	13	103.9	Failed
DIK5243	23	13	105.4	Failed

Appendix I. Amplification of 215 bovine microsatellite markers in the white-tailed deer screening panel.

Marker	Deer LG	Cattle LG	Bovine position (cM)	White-tailed deer amplification
DIK4274	11	11	0.0	Failed
HAUT30	11	11	1.5	Polymorphic after optimization (genotyping failed)
HELMTT43	11	11	2.2	Failed
DIK2715	11	11	3.2	Failed
DIK5318	11	11	5.5	Failed
DIK4735	11	11	8.5	Failed
BM827	11	11	10.6	Failed
BMS2621	11	11	12.1	Monomorphic at standard
DIK4158	11	11	14.1	Polymorphic at standard
BMS424	11	11	14.7	Failed
BMS2131	11	11	18.9	Polymorphic after optimization
BM716	11	11	19.4	Failed
BMS2569	11	11	21.1	Failed
BMS1953	11	11	21.5	Polymorphic after optimization (genotyping failed)
DIK5029	11	11	23.8	Monomorphic at standard
BP38	11	11	24.6	Failed
BM2818	11	11	30.0	Failed
BM304	11	11	33.6	Polymorphic with null allele after optimization
INRA177	11	11	35.1	Polymorphic at standard
DIK5018	11	11	36.4	Polymorphic at standard
CA096	11	11	40.5	Polymorphic after optimization (genotyping failed)
DIK4262	11	11	41.8	Failed
DIK5145	11	11	43.7	Polymorphic after optimization
DIK2027	11	11	46.1	Failed
INRA131	11	11	47.3	Monomorphic after optimization
MS2180	11	11	48.8	Polymorphic after optimization (genotyping failed)
DIK2946	11	11	50.0	Failed
INRA111	11	11	53.1	Polymorphic at standard
UMBTL70	11	11	55.4	Polymorphic after optimization
DIK4796	11	11	56.2	Failed
DIK2894	11	11	59.1	Failed
ILSTS100	11	11	59.1	Polymorphic after optimization
MNS-104	11	11	59.1	Failed
DIK5387	11	11	60.0	Failed
DIK4094	11	11	61.3	Failed
BM6445	11	11	61.6	Polymorphic at standard
ILSTS036	11	11	61.6	Polymorphic after optimization (genotyping failed)
UMBTL184	11	11	61.6	Polymorphic at standard
DIK4541	11	11	63.0	Failed
DIK4675	11	11	63.4	Failed
INRABER169	11	11	65.2	Polymorphic at standard
INRA032	11	11	68.2	Failed
RM150	11	11	70.1	Failed
BMS710	11	11	73.1	Failed
TGLA340	11	11	75.2	Polymorphic after optimization (genotyping failed)
BM8118	11	11	77.1	Monomorphic at standard
BM1861	11	11	79.4	Polymorphic after optimization (genotyping failed)
IDVGA-3	11	11	81.8	Failed
DIK4691	11	11	84.6	Failed
DIK2333	11	11	89.9	Polymorphic at standard
BMS989	11	11	92.2	Polymorphic at standard
DIK4486	11	11	95.9	Failed
BM746	11	11	96.2	Polymorphic at standard

Appendix I. Amplification of 215 bovine microsatellite markers in the white-tailed deer screening panel.

Marker	Deer LG	Cattle LG	Bovine position (cM)	White-tailed deer amplification
BM3501	11	11	97.2	Polymorphic after optimization
TGLA436	11	11	105.2	Monomorphic at standard
BMS460	11	11	109.4	Polymorphic after optimization
CP34	11	11	110.0	Polymorphic at standard
ILSTS028	11	11	112.6	Polymorphic at standard
ILSTS045	11	11	114.2	Failed
BMS655	11	11	117.0	Failed
RM379	11	11	118.8	Failed
DIK5263	11	11	120.5	Polymorphic at standard
DIK5391	11	11	122.4	Failed
DIK4819	11	11	125.0	Monomorphic after optimization
DIK2571	11	11	126.1	Failed
BMS1350	11	11	131.0	Failed
BB718	5	17	0.0	Polymorphic at standard
MNS-101	5	17	1.6	Failed
RM156	5	17	2.4	Failed
URB048	5	17	4.8	Polymorphic at standard
BMS1825	5	17	5.5	Failed
DIK4384	5	17	10.3	Polymorphic at standard
DIK2051	5	17	12.3	Monomorphic at standard
VH98	5	17	15.4	Polymorphic at standard
DIK2105	5	17	18.8	Failed
DIK4665	5	17	21.4	Polymorphic at standard
DIK2858	5	17	25.5	Polymorphic at standard
BB707	5	17	28.7	Failed
INRA193	5	17	33.4	Failed
DIK4122	5	17	34.9	Polymorphic after optimization (genotyping failed)
BMS2780	5	17	36.3	Failed
BMS1373	5	17	38.8	Polymorphic at standard
FCB48	5	17	41.7	Polymorphic at standard
BM305	5	17	44.4	Monomorphic after optimization
BM9138	5	17	46.8	Polymorphic at standard
DIK4218	5	17	52.3	Failed
CP16	5	17	54.7	Polymorphic after optimization
BMS1167	5	17	57.1	Failed
DIK4141	5	17	58.4	Polymorphic after optimization (genotyping failed)
IDVGA-40	5	17	59.6	Failed
DIK4999	5	17	62.4	Monomorphic at standard
DIK2910	5	17	64.0	Failed
CSSM033	5	17	67.3	Failed
DIK5227	5	17	71.4	Monomorphic after optimization
BL50	5	17	72.2	Polymorphic at standard
MNS-96	5	17	73.5	Polymorphic at standard
ILSTS058	5	17	75.4	Polymorphic after optimization (genotyping failed)
DIK4383	5	17	78.1	Polymorphic at standard
UW68	5	17	80.0	Failed
INRA025	5	17	81.8	Failed
DIK2023	5	17	84.1	Polymorphic after optimization
MNB-52	5	17	87.5	Polymorphic after optimization
DIK643	5	17	89.6	Polymorphic at standard
DIK4243	5	17	91.4	Failed
BB1542	5	17	94.0	Polymorphic after optimization
DIK4622	5	17	95.9	Failed

Appendix I. Amplification of 215 bovine microsatellite markers in the white-tailed deer screening panel.

Marker	Deer LG	Cattle LG	Bovine position (cM)	White-tailed deer amplification
BM9202	5	19	0.0	Failed
DIK2574	5	19	0.0	Failed
DIK2200	5	19	3.6	Polymorphic after optimization
BM6000	5	19	5.4	Failed
DIK4341	5	19	11.4	Monomorphic after optimization
DIK2714	5	19	12.6	Failed
X82261	5	19	18.8	Polymorphic after optimization
DIK4582	5	19	21.4	Polymorphic at standard
DIK4932	5	19	23.6	Failed
DIK5289	5	19	26.9	Polymorphic after optimization (genotyping failed)
DIK2067	5	19	27.8	Failed
DIK4009	5	19	33.1	Polymorphic after optimization
DIK5136	5	19	33.7	Polymorphic at standard
DIK5332	5	19	33.7	Polymorphic after optimization
DIK2070	5	19	38.6	Failed
MS2549	5	19	39.0	Failed
RM222	5	19	41.6	Failed
BMS2142	5	19	43.3	Polymorphic at standard
CSSME070	5	19	43.3	Monomorphic at standard
BP20	5	19	45.9	Polymorphic at standard
IDVGA-46	5	19	47.0	Failed
DIK4051	5	19	48.5	Polymorphic at standard
BMS2503	5	19	51.3	Monomorphic at standard
UW40	5	19	53.1	Failed
DIK5188	5	19	55.2	Failed
DIK5098	5	19	58.1	Failed
DIK2722	5	19	61.0	Monomorphic after optimization
DIK4248	5	19	63.8	Failed
DIK2830	5	19	66.8	Monomorphic after optimization
BMS501	5	19	70.2	Polymorphic at standard
DIK4608	5	19	72.3	Failed
DIK4256	5	19	74.8	Polymorphic at standard
IDVGA-48	5	19	76.2	Failed
DIK4611	5	19	78.6	Failed
DIK4394	5	19	82.5	Polymorphic at standard (genotyping failed)
DIK042	5	19	83.5	Failed
NLBCMK40	5	19	83.8	Failed
NLBCMK24	5	19	85.2	Failed
NLBCMK33	5	19	85.6	Monomorphic at standard
IDVGA-44	5	19	86.0	Failed
NLBCMK31	5	19	86.6	Failed
DIK4570	5	19	87.4	Failed
NLBCMK36	5	19	87.4	Monomorphic after optimization
NLBCMK28	5	19	88.1	Failed
NLBCMK29	5	19	88.1	Polymorphic at standard (genotyping failed)
ETH3	5	19	90.0	Failed
DIK4018	5	19	92.0	Failed
DIK4870	5	19	92.4	Failed
DIK1131	5	19	94.2	Failed
DIK4273	5	19	94.2	Monomorphic after optimization
DIK5199	5	19	95.0	Polymorphic at standard (genotyping failed)
BMS2842	5	19	96.5	Failed
DIK4415	5	19	98.5	Polymorphic at standard

Appendix I. Amplification of 215 bovine microsatellite markers in the white-tailed deer screening panel.

Marker	Deer LG	Cattle LG	Bovine position (cM)	White-tailed deer amplification
DIK1119	5	19	100.1	Failed
BMC1013	5	19	106.8	Failed
DIK4898	5	19	109.6	Polymorphic at standard

Information of the bovine microsatellite markers and their original references can be found in the supplemental materials of IHARA *et al.* (2004) <http://genome.cshlp.org/cgi/content/full/14/10a/1987/DC1>. All the markers were initially screened at a standard PCR condition with annealing temperature of 52 °C.

Appendix II. CWD case-control association test results by conditional regression for 53 microsatellite markers in white-tailed deer.

Marker	Allele†	Freq. case	Freq. control	Para-meter	S.E.	χ^2	*P*	Odds ratio	95% CI Lower	95% CI Upper
AGLA232	A164	0.26	0.33	-0.48	0.31	2.47	0.12	0.62	0.34	1.13
(N=192)	A165	0.74	0.67	0.19	0.55	0.11	0.74	1.20	0.41	3.52
BB1542	A5	0.02	0.05	-0.99	0.73	1.83	0.18	0.37	0.09	1.56
(N=184)	A266	0.38	0.32	-0.26	0.43	0.37	0.55	0.77	0.33	1.80
	A276	0.29	0.33	-0.69	0.45	2.34	0.13	0.50	0.21	1.21
	A278	0.21	0.20	-0.15	0.44	0.12	0.73	0.86	0.36	2.05
	A280	0.09	0.07	-0.07	0.51	0.02	0.89	0.93	0.34	2.53
	A288	0.02	0.03	-0.68	0.71	0.92	0.34	0.51	0.13	2.03
BB718	A5	0.10	0.09	0.67	0.60	1.25	0.26	1.96	0.60	6.35
(N=184)	A152	0.26	0.17	1.20	0.61	3.90	0.05	3.31	1.01	10.88
	A154	0.17	0.20	0.59	0.61	0.94	0.33	1.81	0.55	5.99
	A164	0.08	0.14	0.20	0.63	0.11	0.75	1.23	0.36	4.22
	A166	0.13	0.16	0.59	0.62	0.88	0.35	1.80	0.53	6.11
	A168	0.07	0.06	0.86	0.68	1.63	0.20	2.37	0.63	8.92
	A170	0.09	0.08	0.93	0.65	2.06	0.15	2.53	0.71	9.01
	A174	0.11	0.11	0.47	0.61	0.59	0.44	1.60	0.48	5.29
BL1071	A5	0.15	0.14	0.11	0.45	0.06	0.80	1.12	0.46	2.72
(N=184)	A185	0.13	0.13	-0.36	0.50	0.52	0.47	0.70	0.26	1.85
	A191	0.27	0.26	-0.25	0.40	0.38	0.54	0.78	0.35	1.72
	A203	0.02	0.03	-0.51	0.87	0.34	0.56	0.60	0.11	3.30
	A205	0.05	0.08	-0.83	0.58	2.07	0.15	0.44	0.14	1.35
	A207	0.33	0.32	-0.16	0.47	0.12	0.73	0.85	0.34	2.13
	A209	0.06	0.05	-0.11	0.59	0.04	0.85	0.89	0.28	2.86
BL42	A5	0.06	0.08	-0.32	0.58	0.31	0.58	0.72	0.23	2.25
(N=190)	A256	0.31	0.33	-0.22	0.48	0.21	0.65	0.80	0.31	2.07
	A260	0.13	0.17	-0.31	0.44	0.51	0.48	0.73	0.31	1.72
	A262	0.20	0.18	0.10	0.44	0.05	0.82	1.10	0.47	2.60
	A264	0.09	0.08	-0.03	0.51	0.00	0.95	0.97	0.36	2.65
	A266	0.09	0.07	0.05	0.51	0.01	0.93	1.05	0.38	2.86
	A268	0.13	0.09	0.27	0.52	0.27	0.61	1.31	0.47	3.65
BL50	A5	0.07	0.12	-0.63	0.61	1.08	0.30	0.53	0.16	1.75
(N=190)	A227	0.15	0.15	0.07	0.53	0.02	0.89	1.07	0.38	3.01
	A235	0.15	0.21	-0.09	0.47	0.04	0.85	0.91	0.36	2.31
	A237	0.22	0.21	0.00	0.54	0.00	0.99	1.01	0.35	2.87
	A239	0.25	0.19	0.40	0.47	0.73	0.39	1.50	0.59	3.78
	A241	0.16	0.12	0.56	0.50	1.25	0.26	1.75	0.66	4.70
BM6445	A5	0.10	0.08	0.82	0.56	2.10	0.15	2.26	0.75	6.84
(N=192)	A129	0.11	0.13	0.46	0.55	0.68	0.41	1.58	0.54	4.66
	A133	0.15	0.10	0.85	0.54	2.53	0.11	2.35	0.82	6.72
	A137	0.25	0.28	0.39	0.51	0.58	0.45	1.47	0.55	3.96
	A143	0.13	0.16	0.25	0.47	0.27	0.60	1.28	0.51	3.21
	A145	0.07	0.06	0.72	0.61	1.37	0.24	2.05	0.62	6.81
	A147	0.11	0.10	0.69	0.51	1.86	0.17	1.99	0.74	5.37
	A151	0.07	0.08	0.29	0.57	0.26	0.61	1.34	0.44	4.12
BM746	A5	0.05	0.06	0.32	0.58	0.29	0.59	1.37	0.44	4.32
(N=186)	A160	0.09	0.11	0.08	0.54	0.02	0.88	1.08	0.37	3.13
	A164	0.13	0.17	-0.10	0.52	0.04	0.84	0.90	0.32	2.51
	A166	0.11	0.08	0.60	0.48	1.52	0.22	1.82	0.70	4.69
	A172	0.36	0.39	0.40	0.53	0.58	0.45	1.50	0.53	4.20
	A174	0.08	0.05	0.88	0.61	2.09	0.15	2.40	0.73	7.90
	A178	0.09	0.04	1.14	0.57	4.00	0.05	3.12	1.02	9.52
	A180	0.09	0.11	0.22	0.54	0.16	0.69	1.24	0.43	3.59
BMS1226	A5	0.06	0.06	0.47	0.58	0.66	0.41	1.60	0.52	4.98

Appendix II. CWD case-control association test results by conditional regression for 53 microsatellite markers in white-tailed deer.

Marker	Allele†	Freq. case	Freq. control	Para-meter	S.E.	χ^2	*P*	Odds ratio	95% CI Lower	95% CI Upper
(N=186)	A162	0.22	0.19	0.59	0.50	1.40	0.24	1.80	0.68	4.75
	A164	0.11	0.06	0.81	0.58	1.98	0.16	2.26	0.73	7.01
	A166	0.16	0.20	0.07	0.53	0.02	0.90	1.07	0.38	3.04
	A168	0.11	0.12	0.22	0.53	0.18	0.68	1.25	0.45	3.49
	A172	0.07	0.08	0.08	0.57	0.02	0.89	1.08	0.35	3.29
	A176	0.13	0.10	0.55	0.53	1.05	0.30	1.73	0.61	4.90
	A178	0.08	0.09	0.14	0.51	0.08	0.78	1.16	0.42	3.16
	A180	0.06	0.09	0.04	0.57	0.01	0.94	1.04	0.35	3.16
BMS1373	A115	0.13	0.17	-0.55	0.44	1.59	0.21	0.58	0.25	1.36
(N=192)	A116	0.08	0.08	-0.12	0.49	0.06	0.80	0.88	0.34	2.29
	A118	0.20	0.12	0.41	0.37	1.22	0.27	1.51	0.73	3.14
	A120	0.59	0.64	-0.38	0.53	0.51	0.47	0.68	0.24	1.94
BMS1669*	A5	0.09	0.11	0.74	0.60	1.52	0.22	2.09	0.65	6.74
(N=190)	A109	0.08	0.06	1.27	0.65	3.84	0.05	3.56	1.00	12.67
	A113	0.22	0.15	1.08	0.47	5.20	0.02	2.94	1.16	7.45
	A121	0.18	0.12	1.55	0.63	6.00	0.01	4.71	1.36	16.25
	A123	0.05	0.12	-0.32	0.54	0.37	0.55	0.72	0.25	2.07
	A125	0.23	0.29	0.55	0.52	1.12	0.29	1.74	0.62	4.86
	A127	0.06	0.11	0.05	0.60	0.01	0.93	1.05	0.33	3.38
	A131	0.08	0.05	1.46	0.69	4.54	0.03	4.32	1.12	16.61
BMS2131	A5	0.12	0.10	0.25	0.48	0.28	0.60	1.29	0.50	3.29
(N=186)	A230	0.42	0.41	-0.04	0.47	0.01	0.94	0.96	0.39	2.40
	A232	0.19	0.15	0.32	0.42	0.56	0.46	1.37	0.60	3.15
	A236	0.05	0.05	-0.04	0.51	0.01	0.94	0.97	0.36	2.62
	A240	0.08	0.11	-0.29	0.46	0.40	0.53	0.75	0.30	1.85
	A242	0.04	0.06	-0.58	0.66	0.79	0.37	0.56	0.15	2.02
	A244	0.10	0.13	-0.05	0.45	0.01	0.91	0.95	0.39	2.30
BMS2142	A5	0.30	0.24	-0.12	0.43	0.07	0.79	0.89	0.38	2.07
(N=188)	A135	0.30	0.31	-0.33	0.45	0.54	0.46	0.72	0.30	1.73
	A136	0.14	0.16	-0.49	0.42	1.35	0.25	0.61	0.27	1.40
	A137	0.13	0.16	-0.48	0.45	1.15	0.28	0.62	0.26	1.49
	A139	0.11	0.09	-0.04	0.43	0.01	0.92	0.96	0.41	2.24
	A141	0.03	0.03	0.03	0.69	0.00	0.97	1.03	0.26	3.99
BMS2319	A5	0.07	0.09	-0.22	0.47	0.21	0.65	0.81	0.32	2.03
(N=192)	A112	0.35	0.39	0.03	0.43	0.01	0.94	1.03	0.45	2.39
	A122	0.06	0.05	0.31	0.53	0.34	0.56	1.36	0.48	3.82
	A124	0.05	0.05	-0.04	0.59	0.00	0.95	0.97	0.30	3.08
	A132	0.40	0.35	0.42	0.43	0.95	0.33	1.52	0.65	3.55
	A134	0.08	0.07	0.20	0.47	0.17	0.68	1.22	0.48	3.08
BMS460	A5	0.28	0.24	-0.65	0.47	1.90	0.17	0.52	0.21	1.31
(N=192)	A145	0.10	0.12	-0.57	0.52	1.21	0.27	0.57	0.21	1.56
	A146	0.04	0.06	-1.01	0.75	1.80	0.18	0.36	0.08	1.60
	A150	0.13	0.12	-0.38	0.51	0.54	0.46	0.69	0.25	1.87
	A152	0.13	0.09	0.13	0.52	0.06	0.80	1.14	0.41	3.15
	A154	0.11	0.13	-0.61	0.54	1.26	0.26	0.55	0.19	1.57
	A158	0.06	0.11	-1.14	0.57	4.03	0.04	0.32	0.10	0.97
	A162	0.16	0.13	-0.16	0.54	0.08	0.77	0.86	0.30	2.45
BMS501	A5	0.15	0.10	0.97	0.53	3.38	0.07	2.65	0.94	7.46
(N=192)	A134	0.05	0.05	0.51	0.62	0.68	0.41	1.67	0.50	5.59
	A138	0.13	0.09	0.99	0.57	3.04	0.08	2.69	0.89	8.17
	A142	0.26	0.32	-0.17	0.47	0.13	0.72	0.84	0.34	2.12
	A144	0.09	0.11	0.07	0.52	0.02	0.89	1.07	0.39	2.96
	A146	0.07	0.07	0.21	0.56	0.14	0.71	1.24	0.41	3.74
	A154	0.09	0.10	-0.05	0.50	0.01	0.92	0.95	0.36	2.56

Appendix II. CWD case-control association test results by conditional regression for 53 microsatellite markers in white-tailed deer.

Marker	Allele†	Freq. case	Freq. control	Para-meter	S.E.	χ^2	*P*	Odds ratio	95% CI Lower	95% CI Upper
	A158	0.09	0.09	0.00	0.55	0.00	0.99	1.00	0.34	2.91
	A160	0.06	0.06	0.34	0.58	0.34	0.56	1.40	0.45	4.36
BMS989	A5	0.11	0.06	1.36	0.65	4.33	0.04	3.90	1.08	14.04
(N=190)	A111	0.10	0.08	0.92	0.62	2.19	0.14	2.50	0.74	8.40
	A113	0.08	0.07	0.59	0.56	1.09	0.30	1.80	0.60	5.44
	A115	0.20	0.21	0.61	0.60	1.05	0.31	1.84	0.57	5.94
	A121	0.19	0.24	0.28	0.60	0.21	0.65	1.32	0.41	4.30
	A123	0.13	0.15	0.06	0.62	0.01	0.93	1.06	0.31	3.56
	A125	0.08	0.09	0.23	0.61	0.15	0.70	1.26	0.38	4.19
	A129	0.09	0.10	0.48	0.62	0.60	0.44	1.62	0.48	5.48
BP20	A224	0.74	0.72	0.95	0.70	1.85	0.17	2.59	0.66	10.16
(N=190)	A226	0.21	0.22	0.28	0.33	0.71	0.40	1.33	0.69	2.55
	A227	0.05	0.06	0.12	0.60	0.04	0.84	1.13	0.35	3.67
CP16	A5	0.22	0.19	0.57	0.50	1.31	0.25	1.77	0.67	4.69
(N=190)	A100	0.21	0.23	0.50	0.47	1.12	0.29	1.65	0.65	4.19
	A102	0.19	0.16	0.71	0.51	1.89	0.17	2.03	0.74	5.55
	A110	0.07	0.10	-0.06	0.44	0.02	0.89	0.94	0.39	2.25
	A112	0.06	0.09	-0.07	0.51	0.02	0.89	0.93	0.34	2.55
	A114	0.25	0.23	0.45	0.43	1.12	0.29	1.58	0.68	3.65
CP34	A5	0.18	0.16	0.25	0.47	0.29	0.59	1.29	0.51	3.26
(N=192)	A125	0.09	0.10	-0.24	0.48	0.25	0.62	0.79	0.31	2.01
	A129	0.15	0.19	-0.34	0.47	0.53	0.47	0.71	0.28	1.78
	A131	0.21	0.24	-0.18	0.46	0.16	0.69	0.83	0.34	2.05
	A133	0.36	0.30	0.09	0.45	0.04	0.84	1.10	0.45	2.66
DIK2023	A5	0.06	0.07	0.31	0.55	0.31	0.58	1.36	0.46	4.03
(N=192)	A171	0.08	0.05	1.07	0.62	3.00	0.08	2.91	0.87	9.75
	A177	0.07	0.04	1.14	0.66	3.00	0.08	3.14	0.86	11.44
	A179	0.15	0.14	0.79	0.57	1.90	0.17	2.21	0.72	6.78
	A181	0.15	0.15	0.39	0.55	0.49	0.48	1.47	0.50	4.35
	A183	0.07	0.06	0.82	0.60	1.85	0.17	2.26	0.70	7.32
	A185	0.08	0.13	0.01	0.58	0.00	0.98	1.01	0.33	3.13
	A187	0.16	0.19	0.31	0.58	0.29	0.59	1.37	0.44	4.28
	A189	0.17	0.18	0.61	0.50	1.47	0.23	1.84	0.69	4.93
DIK2058	A168	0.43	0.42	0.04	0.47	0.01	0.94	1.04	0.41	2.60
(N=190)	A170	0.12	0.14	-0.22	0.45	0.23	0.63	0.81	0.34	1.94
	A172	0.37	0.31	0.11	0.38	0.08	0.77	1.12	0.53	2.34
	A174	0.09	0.13	-0.37	0.43	0.73	0.39	0.69	0.30	1.62
DIK2117	A5	0.19	0.15	0.40	0.49	0.65	0.42	1.49	0.57	3.90
(N=184)	A220	0.36	0.37	0.29	0.50	0.33	0.57	1.33	0.50	3.55
	A224	0.05	0.08	-0.57	0.60	0.92	0.34	0.56	0.17	1.82
	A230	0.17	0.21	-0.09	0.46	0.04	0.84	0.91	0.37	2.26
	A232	0.10	0.10	0.07	0.43	0.02	0.88	1.07	0.46	2.50
	A239	0.04	0.05	-0.09	0.59	0.02	0.88	0.92	0.29	2.92
	A240	0.08	0.04	0.71	0.62	1.29	0.26	2.02	0.60	6.84
DIK2333	A5	0.15	0.08	0.59	0.46	1.63	0.20	1.80	0.73	4.44
(N=190)	A203	0.49	0.53	-0.16	0.48	0.11	0.74	0.85	0.33	2.19
	A205	0.09	0.08	-0.23	0.50	0.21	0.65	0.79	0.30	2.14
	A207	0.12	0.13	-0.33	0.45	0.56	0.46	0.72	0.30	1.72
	A209	0.07	0.06	0.15	0.55	0.08	0.78	1.16	0.40	3.42
	A219	0.07	0.12	-0.55	0.45	1.49	0.22	0.58	0.24	1.39
DIK2858	A5	0.21	0.17	0.19	0.39	0.23	0.63	1.21	0.56	2.57
(N=190)	A206	0.23	0.22	0.04	0.33	0.01	0.91	1.04	0.55	1.97

Appendix II. CWD case-control association test results by conditional regression for 53 microsatellite markers in white-tailed deer.

Marker	Allele†	Freq. case	Freq. control	Para-meter	S.E.	χ^2	*P*	Odds ratio	95% CI Lower	95% CI Upper
	A208	0.56	0.61	-0.50	0.46	1.18	0.28	0.61	0.25	1.49
DIK4009	A5	0.12	0.12	-0.08	0.55	0.02	0.88	0.92	0.32	2.69
(N=190)	A194	0.10	0.06	0.39	0.57	0.46	0.50	1.48	0.48	4.55
	A200	0.08	0.04	0.68	0.66	1.04	0.31	1.97	0.54	7.24
	A202	0.06	0.08	-0.67	0.76	0.78	0.38	0.51	0.12	2.27
	A204	0.05	0.06	-0.33	0.74	0.20	0.65	0.72	0.17	3.07
	A206	0.18	0.17	0.07	0.52	0.02	0.90	1.07	0.39	2.95
	A208	0.11	0.15	-0.44	0.59	0.57	0.45	0.64	0.20	2.03
	A212	0.08	0.08	0.01	0.60	0.00	0.98	1.01	0.31	3.28
	A216	0.12	0.17	-0.01	0.59	0.00	0.98	0.99	0.31	3.12
	A218	0.09	0.06	0.10	0.59	0.03	0.87	1.10	0.35	3.52
DIK4051	A5	0.08	0.07	0.04	0.56	0.01	0.94	1.04	0.35	3.13
(N=188)	A223	0.31	0.28	-0.16	0.52	0.09	0.76	0.85	0.31	2.37
	A225	0.05	0.10	-0.81	0.60	1.83	0.18	0.44	0.14	1.44
	A227	0.16	0.13	0.28	0.54	0.28	0.60	1.33	0.46	3.83
	A229	0.09	0.08	-0.07	0.52	0.02	0.89	0.93	0.33	2.59
	A235	0.10	0.11	0.00	0.55	0.00	1.00	1.00	0.34	2.95
	A237	0.16	0.19	-0.29	0.46	0.39	0.53	0.75	0.30	1.86
	A239	0.05	0.05	0.14	0.66	0.04	0.83	1.15	0.31	4.23
DIK4158	A5	0.17	0.20	0.62	0.59	1.12	0.29	1.87	0.59	5.92
(N=188)	A237	0.15	0.12	0.92	0.59	2.42	0.12	2.51	0.79	7.98
	A243	0.18	0.17	0.77	0.57	1.81	0.18	2.15	0.71	6.56
	A245	0.06	0.05	0.77	0.63	1.51	0.22	2.16	0.63	7.36
	A247	0.13	0.09	0.78	0.56	1.98	0.16	2.18	0.74	6.48
	A249	0.08	0.10	0.37	0.58	0.40	0.53	1.44	0.46	4.53
	A255	0.13	0.13	0.42	0.57	0.53	0.47	1.52	0.50	4.63
	A265	0.11	0.15	0.15	0.58	0.06	0.80	1.16	0.37	3.62
DIK4242	A5	0.13	0.18	-0.38	0.43	0.77	0.38	0.69	0.30	1.59
(N=186)	A269	0.32	0.25	0.31	0.40	0.60	0.44	1.36	0.62	2.98
	A275	0.06	0.04	0.12	0.56	0.04	0.84	1.12	0.37	3.38
	A277	0.06	0.04	0.29	0.63	0.22	0.64	1.34	0.39	4.64
	A279	0.08	0.08	-0.25	0.55	0.21	0.65	0.78	0.27	2.28
	A281	0.11	0.10	0.00	0.47	0.00	1.00	1.00	0.40	2.49
	A283	0.09	0.10	0.00	-	-	-	-	-	-
	A295	0.15	0.20	-0.56	0.40	1.96	0.16	0.57	0.26	1.25
DIK4256	A5	0.04	0.08	-0.69	0.67	1.05	0.31	0.50	0.13	1.88
(N=192)	A234	0.19	0.16	0.37	0.56	0.43	0.51	1.45	0.48	4.33
	A240	0.14	0.14	-0.05	0.61	0.01	0.93	0.95	0.29	3.16
	A250	0.07	0.09	-0.11	0.65	0.03	0.86	0.89	0.25	3.19
	A252	0.24	0.21	0.25	0.56	0.21	0.65	1.29	0.43	3.85
	A254	0.16	0.18	-0.11	0.61	0.03	0.85	0.89	0.27	2.93
	A256	0.16	0.14	0.28	0.53	0.28	0.59	1.33	0.47	3.77
DIK4358	A5	0.15	0.17	-0.49	0.58	0.69	0.41	0.62	0.20	1.94
(N=190)	A132	0.15	0.12	0.22	0.46	0.22	0.64	1.24	0.50	3.08
	A134	0.25	0.20	-0.10	0.51	0.04	0.84	0.91	0.34	2.45
	A136	0.04	0.06	-0.46	0.59	0.60	0.44	0.63	0.20	2.02
	A138	0.09	0.12	-0.44	0.54	0.65	0.42	0.65	0.22	1.88
	A142	0.08	0.06	-0.11	0.55	0.04	0.84	0.90	0.31	2.62
	A148	0.08	0.10	-0.49	0.51	0.93	0.33	0.61	0.23	1.66
	A150	0.15	0.17	-0.60	0.53	1.30	0.25	0.55	0.19	1.54
DIK4384	A5	0.07	0.11	-0.81	0.56	2.09	0.15	0.44	0.15	1.34
(N=190)	A224	0.05	0.09	-1.07	0.66	2.61	0.11	0.34	0.09	1.26
	A226	0.05	0.04	-0.31	0.68	0.21	0.65	0.73	0.19	2.79
	A228	0.27	0.28	-0.34	0.51	0.44	0.51	0.71	0.26	1.94

Appendix II. CWD case-control association test results by conditional regression for 53 microsatellite markers in white-tailed deer.

Marker	Allele†	Freq. case	Freq. control	Para-meter	S.E.	χ^2	*P*	Odds ratio	95% CI Lower	95% CI Upper
	A230	0.10	0.11	-0.03	0.57	0.00	0.96	0.97	0.32	2.95
	A232	0.08	0.11	-0.77	0.58	1.74	0.19	0.46	0.15	1.45
	A242	0.16	0.14	0.27	0.52	0.27	0.60	1.31	0.47	3.60
	A244	0.13	0.07	0.64	0.56	1.31	0.25	1.89	0.64	5.63
	A246	0.09	0.04	0.89	0.61	2.16	0.14	2.44	0.74	8.04
DIK4582	A219	0.06	0.04	0.23	0.52	0.19	0.66	1.26	0.45	3.52
(N=190)	A229	0.59	0.63	-0.68	0.51	1.80	0.18	0.51	0.19	1.37
	A231	0.26	0.25	-0.10	0.31	0.10	0.75	0.91	0.49	1.68
	A233	0.08	0.09	-0.18	0.42	0.19	0.66	0.83	0.37	1.89
DIK4665	A5	0.17	0.13	0.52	0.50	1.07	0.30	1.68	0.63	4.49
(N=190)	A200	0.04	0.07	-0.84	0.66	1.61	0.20	0.43	0.12	1.58
	A202	0.14	0.19	-0.39	0.51	0.57	0.45	0.68	0.25	1.85
	A214	0.28	0.27	0.24	0.48	0.25	0.62	1.27	0.50	3.27
	A216	0.07	0.10	-0.44	0.56	0.61	0.43	0.65	0.22	1.93
	A218	0.19	0.21	0.26	0.46	0.32	0.57	1.29	0.53	3.16
	A228	0.11	0.03	1.17	0.61	3.66	0.06	3.24	0.97	10.79
DIK5018	A218	0.26	0.25	-0.11	0.45	0.06	0.81	0.90	0.38	2.16
(N=192)	A222	0.05	0.07	-0.38	0.57	0.45	0.50	0.69	0.23	2.08
	A223	0.06	0.08	-0.40	0.51	0.62	0.43	0.67	0.25	1.81
	A224	0.40	0.36	0.14	0.45	0.10	0.76	1.15	0.48	2.78
	A226	0.17	0.16	-0.04	0.42	0.01	0.92	0.96	0.42	2.21
	A228	0.08	0.08	-0.06	0.47	0.02	0.90	0.94	0.37	2.37
DIK5136	A5	0.09	0.07	-0.31	0.51	0.37	0.54	0.73	0.27	1.99
(N=190)	A260	0.46	0.58	-1.17	0.52	5.13	0.02	0.31	0.11	0.85
	A262	0.22	0.15	-0.01	0.39	0.00	0.98	0.99	0.46	2.13
	A264	0.09	0.09	-0.23	0.45	0.27	0.60	0.79	0.33	1.92
	A266	0.14	0.11	-0.12	0.39	0.09	0.76	0.89	0.41	1.92
DIK5145	A5	0.26	0.26	0.10	0.37	0.08	0.78	1.11	0.54	2.26
(N=190)	A182	0.32	0.41	-0.52	0.39	1.82	0.18	0.59	0.28	1.27
	A184	0.42	0.34	0.30	0.36	0.68	0.41	1.35	0.66	2.74
DIK643	A5	0.08	0.09	-0.10	0.50	0.04	0.84	0.91	0.34	2.41
(N=188)	A179	0.06	0.08	-0.53	0.52	1.05	0.31	0.59	0.22	1.62
	A181	0.43	0.36	0.12	0.42	0.08	0.77	1.13	0.49	2.59
	A183	0.20	0.17	0.20	0.39	0.26	0.61	1.22	0.56	2.64
	A185	0.23	0.30	-0.35	0.40	0.75	0.39	0.71	0.32	1.55
DIK708	A208	0.96	0.94	14.07	1214	0.00	0.99	>999	<0.001	>999
(N=192)	A209	0.04	0.06	-0.13	0.52	0.07	0.80	0.88	0.32	2.41
HUJ616	A5	0.11	0.18	0.20	0.43	0.21	0.64	1.22	0.52	2.86
(N=190)	A126	0.26	0.23	0.79	0.46	3.00	0.08	2.20	0.90	5.38
	A132	0.24	0.21	1.01	0.48	4.43	0.04	2.75	1.07	7.04
	A136	0.21	0.20	0.42	0.42	1.01	0.31	1.52	0.67	3.44
	A138	0.18	0.17	0.64	0.46	1.96	0.16	1.89	0.78	4.61
ILSTS028	A5	0.04	0.05	-0.12	0.49	0.06	0.81	0.89	0.34	2.30
(N=192)	A151	0.96	0.95	0.00	-	-	-	-	-	-
ILSTS059	A5	0.06	0.05	-0.47	0.69	0.47	0.49	0.62	0.16	2.40
(N=192)	A167	0.08	0.14	-0.88	0.54	2.73	0.10	0.41	0.15	1.18
	A169	0.35	0.23	0.26	0.45	0.33	0.57	1.30	0.53	3.15
	A171	0.06	0.08	-0.62	0.59	1.11	0.29	0.54	0.17	1.71
	A173	0.36	0.39	-0.59	0.54	1.19	0.28	0.56	0.19	1.60
	A179	0.09	0.11	-0.55	0.50	1.19	0.28	0.58	0.22	1.55

Appendix II. CWD case-control association test results by conditional regression for 53 microsatellite markers in white-tailed deer.

Marker	Allele†	Freq. case	Freq. control	Para-meter	S.E.	χ^2	*P*	Odds ratio	95% CI Lower	95% CI Upper
ILSTS100	A204	0.94	0.97	0.00	-	-	-	-	-	-
(N=192)	A206	0.06	0.03	0.61	0.51	1.43	0.23	1.83	0.68	4.96
INRA111	A5	0.17	0.19	-0.26	0.42	0.38	0.54	0.77	0.34	1.76
(N=192)	A129	0.14	0.11	0.24	0.46	0.27	0.61	1.27	0.52	3.12
	A131	0.27	0.30	-0.17	0.40	0.19	0.67	0.84	0.39	1.84
	A137	0.14	0.10	0.17	0.45	0.14	0.71	1.18	0.49	2.85
	A143	0.29	0.30	-0.22	0.42	0.28	0.60	0.80	0.35	1.84
INRA177	A5	0.09	0.10	0.05	0.55	0.01	0.94	1.05	0.35	3.10
(N=188)	A92	0.16	0.15	0.27	0.63	0.18	0.67	1.31	0.38	4.47
	A94	0.08	0.11	-0.26	0.54	0.22	0.64	0.77	0.27	2.24
	A96	0.09	0.08	0.25	0.58	0.19	0.66	1.29	0.41	4.06
	A98	0.14	0.11	0.40	0.57	0.49	0.48	1.50	0.49	4.59
	A102	0.13	0.13	-0.03	0.61	0.00	0.96	0.97	0.29	3.22
	A108	0.16	0.14	0.33	0.53	0.38	0.54	1.39	0.49	3.96
	A110	0.06	0.07	-0.44	0.62	0.51	0.48	0.64	0.19	2.17
	A112	0.10	0.11	0.11	0.57	0.03	0.85	1.11	0.37	3.37
INRABER 169	A5	0.08	0.09	0.15	0.52	0.09	0.77	1.16	0.42	3.20
(N=184)	A207	0.35	0.40	0.26	0.52	0.25	0.62	1.30	0.47	3.61
	A220	0.16	0.17	0.31	0.49	0.40	0.53	1.37	0.52	3.61
	A221	0.10	0.07	0.70	0.53	1.77	0.18	2.02	0.72	5.65
	A228	0.05	0.07	0.10	0.54	0.03	0.86	1.10	0.39	3.15
	A232	0.08	0.05	0.59	0.55	1.15	0.28	1.81	0.61	5.34
	A234	0.17	0.15	0.42	0.47	0.81	0.37	1.52	0.61	3.78
MNB-52	A185	0.23	0.23	-0.19	0.33	0.33	0.57	0.83	0.44	1.58
(N=192)	A187	0.77	0.77	-0.65	0.77	0.71	0.40	0.52	0.12	2.37
MNB-77	A216	0.04	0.08	-0.91	0.49	3.40	0.07	0.40	0.15	1.06
(N=192)	A220	0.06	0.04	0.04	0.52	0.01	0.94	1.04	0.37	2.90
	A222	0.90	0.88	-15.42	1318	0.00	0.99	<0.001	<0.001	>999
MNS-96	A5	0.04	0.07	-0.45	0.48	0.87	0.35	0.64	0.25	1.64
(N=190)	A184	0.96	0.93	13.75	1214	0.00	0.99	>999	<0.001	>999
MNS-97	A5	0.07	0.05	0.06	0.45	0.02	0.89	1.06	0.44	2.56
(N=190)	A269	0.08	0.15	-0.91	0.42	4.62	0.03	0.40	0.18	0.92
	A275	0.05	0.06	-0.33	0.55	0.36	0.55	0.72	0.25	2.11
	A277	0.79	0.73	-0.12	0.78	0.02	0.88	0.89	0.19	4.05
UMBTL70	A5	0.23	0.19	0.22	0.62	0.13	0.72	1.25	0.37	4.16
(N=182)	A183	0.18	0.23	-0.51	0.60	0.74	0.39	0.60	0.19	1.93
	A185	0.16	0.15	0.27	0.59	0.21	0.65	1.31	0.41	4.15
	A187	0.07	0.07	-0.20	0.66	0.09	0.77	0.82	0.23	3.00
	A191	0.07	0.05	-0.13	0.69	0.03	0.86	0.88	0.23	3.40
	A193	0.08	0.07	0.01	0.63	0.00	0.99	1.01	0.29	3.48
	A201	0.22	0.23	-0.33	0.56	0.35	0.55	0.72	0.24	2.14
URB021B	A5	0.11	0.08	0.36	0.47	0.60	0.44	1.44	0.57	3.61
(N=192)	A145	0.33	0.40	-0.13	0.45	0.09	0.77	0.88	0.36	2.11
	A147	0.29	0.20	0.55	0.47	1.40	0.24	1.74	0.70	4.33
	A149	0.15	0.16	-0.03	0.48	0.01	0.94	0.97	0.37	2.50
	A151	0.12	0.16	-0.30	0.47	0.40	0.53	0.74	0.30	1.87
URB048	A5	0.24	0.35	-0.05	0.50	0.01	0.93	0.96	0.36	2.54
(N=192)	A189	0.06	0.07	-0.06	0.59	0.01	0.91	0.94	0.30	2.96
	A190	0.09	0.07	0.52	0.53	0.98	0.32	1.69	0.60	4.72
	A194	0.17	0.16	0.25	0.47	0.28	0.60	1.28	0.51	3.23

Appendix II. CWD case-control association test results by conditional regression for 53 microsatellite markers in white-tailed deer.

Marker	Allele†	Freq. case	Freq. control	Para-meter	S.E.	χ^2	*P*	Odds ratio	95% CI Lower	95% CI Upper
	A195	0.15	0.12	0.47	0.50	0.90	0.34	1.60	0.60	4.27
	A196	0.14	0.10	0.70	0.51	1.87	0.17	2.01	0.74	5.45
	A198	0.10	0.07	0.56	0.51	1.22	0.27	1.75	0.65	4.74
	A200	0.05	0.06	0.09	0.57	0.02	0.88	1.09	0.36	3.35

Maximum likelihood estimation of conditional logistic regression parameters and odds ratios were calculated in SAS v9.2. Chi-square (df = 1) and CI were calculated using Wald statistics.

* Indicates markers that were significant in global tests ($\alpha = 0.05$).

† Pooled rare alleles (< 0.05) are denoted as A5.

Appendix III. CWD case-control association test results by conditional regression for 42 microsatellite markers in mule deer.

Marker	Allele†	Freq. case	Freq. control	Para-meter	S.E.	χ^2	*P*	Odds ratio	95% CI Lower	95% CI Upper
BB1542	A5	0.15	0.08	0.92	0.48	3.66	0.06	2.52	0.98	6.49
(N=166)	A275	0.42	0.49	0.03	0.55	0.00	0.96	1.03	0.35	3.05
	A283	0.11	0.11	0.37	0.54	0.47	0.49	1.44	0.51	4.12
	A289	0.05	0.05	0.29	0.65	0.20	0.66	1.33	0.37	4.75
	A295	0.12	0.14	0.18	0.49	0.14	0.71	1.20	0.46	3.16
	A297	0.15	0.13	0.42	0.43	0.96	0.33	1.52	0.66	3.51
BB718	A5	0.06	0.08	-0.28	0.59	0.23	0.63	0.75	0.24	2.39
(N=172)	A155	0.09	0.10	-0.15	0.56	0.07	0.79	0.86	0.29	2.57
	A159	0.37	0.27	0.18	0.52	0.11	0.74	1.19	0.43	3.33
	A169	0.08	0.08	-0.05	0.53	0.01	0.92	0.95	0.34	2.66
	A175	0.08	0.05	0.50	0.57	0.79	0.37	1.66	0.54	5.04
	A177	0.15	0.20	-0.43	0.51	0.73	0.39	0.65	0.24	1.75
	A179	0.17	0.22	-0.42	0.49	0.73	0.39	0.66	0.25	1.72
BL1071	A5	0.09	0.09	0.17	0.41	0.17	0.68	1.18	0.53	2.64
(N=172)	A186	0.91	0.91	14.37	1213	0.00	0.99	>999	<0.001	>999
BL42	A5	0.10	0.08	0.51	0.45	1.29	0.26	1.66	0.69	3.99
(N=166)	A258	0.16	0.17	0.32	0.41	0.61	0.44	1.38	0.62	3.06
	A260	0.74	0.75	0.68	0.65	1.08	0.30	1.97	0.55	7.04
BL50	A5	0.05	0.08	-0.47	0.56	0.70	0.40	0.63	0.21	1.88
(N=168)	A236	0.13	0.10	0.37	0.45	0.66	0.42	1.44	0.60	3.50
	A238	0.45	0.50	0.23	0.51	0.21	0.65	1.26	0.46	3.45
	A240	0.22	0.14	0.76	0.46	2.75	0.10	2.13	0.87	5.22
	A242	0.15	0.18	0.14	0.44	0.11	0.74	1.15	0.49	2.73
BM3501	A5	0.11	0.11	0.09	0.41	0.05	0.83	1.09	0.49	2.47
(N=166)	A184	0.70	0.77	0.29	0.78	0.14	0.70	1.34	0.29	6.15
	A190	0.19	0.11	0.70	0.46	2.29	0.13	2.01	0.81	4.95
BM6445	A5	0.07	0.06	0.98	0.70	1.99	0.16	2.68	0.68	10.52
(N=170)	A130	0.35	0.33	0.94	0.56	2.85	0.09	2.56	0.86	7.66
	A144	0.22	0.16	1.19	0.57	4.39	0.04	3.28	1.08	9.95
	A146	0.16	0.23	0.25	0.56	0.20	0.66	1.29	0.43	3.89
	A148	0.20	0.22	0.33	0.52	0.41	0.52	1.40	0.50	3.86
BMS1226	A5	0.11	0.12	0.09	0.46	0.04	0.84	1.10	0.45	2.69
(N=168)	A159	0.26	0.36	-0.08	0.42	0.03	0.85	0.93	0.41	2.09
	A169	0.36	0.31	0.90	0.47	3.69	0.05	2.46	0.98	6.14
	A171	0.28	0.21	0.93	0.46	4.12	0.04	2.53	1.03	6.18
BMS1669	A5	0.12	0.09	0.71	0.81	0.77	0.38	2.04	0.42	10.01
(N=170)	A120	0.04	0.10	-1.02	0.75	1.87	0.17	0.36	0.08	1.56
	A122	0.19	0.14	0.70	0.76	0.85	0.36	2.01	0.45	8.94
	A132	0.06	0.14	-0.74	0.83	0.80	0.37	0.48	0.09	2.43
	A134	0.18	0.15	0.54	0.70	0.60	0.44	1.72	0.44	6.78
	A136	0.09	0.12	0.21	0.69	0.09	0.76	1.24	0.32	4.82
	A138	0.08	0.09	-0.04	0.75	0.00	0.96	0.96	0.22	4.20
	A140	0.15	0.13	0.35	0.71	0.25	0.62	1.42	0.36	5.71
	A144	0.09	0.05	0.40	0.84	0.23	0.63	1.49	0.29	7.79
BMS2131	A5	0.03	0.06	-1.12	0.76	2.14	0.14	0.33	0.07	1.46
(N=172)	A242	0.06	0.11	-0.71	0.48	2.17	0.14	0.49	0.19	1.26
	A244	0.11	0.16	-0.34	0.52	0.43	0.51	0.71	0.26	1.97
	A246	0.18	0.13	0.24	0.47	0.25	0.62	1.27	0.50	3.20
	A248	0.42	0.35	0.11	0.54	0.04	0.84	1.11	0.39	3.20
	A250	0.13	0.12	-0.12	0.48	0.07	0.80	0.88	0.34	2.28
	A253	0.06	0.06	0.17	0.68	0.06	0.81	1.18	0.31	4.48

Appendix III. CWD case-control association test results by conditional regression for 42 microsatellite markers in mule deer.

Marker	Allele†	Freq. case	Freq. control	Para-meter	S.E.	χ^2	*P*	Odds ratio	95% CI Lower	95% CI Upper
BMS2142	A5	0.05	0.08	-1.06	0.58	3.37	0.07	0.35	0.11	1.07
(N=172)	A130	0.76	0.76	-1.65	0.89	3.45	0.06	0.19	0.03	1.09
	A136	0.12	0.09	-0.14	0.48	0.09	0.76	0.87	0.34	2.22
	A138	0.07	0.07	-0.45	0.50	0.83	0.36	0.64	0.24	1.68
BMS501	A5	0.05	0.09	-0.70	0.60	1.36	0.24	0.50	0.15	1.61
(N=166)	A144	0.41	0.39	0.32	0.53	0.36	0.55	1.37	0.49	3.86
	A146	0.22	0.23	-0.09	0.47	0.04	0.85	0.92	0.36	2.31
	A150	0.19	0.16	0.32	0.48	0.45	0.50	1.38	0.54	3.52
	A158	0.05	0.07	-0.55	0.62	0.78	0.38	0.58	0.17	1.95
	A162	0.07	0.07	0.22	0.57	0.15	0.70	1.25	0.41	3.80
BMS989	A5	0.11	0.11	0.66	0.51	1.67	0.20	1.93	0.71	5.23
(N=166)	A124	0.45	0.45	1.07	0.51	4.49	0.03	2.92	1.08	7.89
	A128	0.22	0.23	0.66	0.46	2.06	0.15	1.93	0.79	4.76
	A130	0.14	0.15	0.55	0.40	1.86	0.17	1.74	0.79	3.83
	A132	0.07	0.05	0.93	0.58	2.56	0.11	2.54	0.81	7.92
CP16	A5	0.08	0.13	-0.57	0.46	1.50	0.22	0.57	0.23	1.41
(N=166)	A104	0.24	0.20	0.03	0.41	0.01	0.94	1.03	0.46	2.30
	A106	0.34	0.34	-0.17	0.41	0.17	0.68	0.84	0.38	1.89
	A114	0.34	0.33	0.05	0.41	0.02	0.89	1.06	0.47	2.36
CP34	A5	0.05	0.05	-0.43	0.64	0.45	0.50	0.65	0.19	2.29
(N=170)	A115	0.04	0.09	-0.94	0.54	3.02	0.08	0.39	0.13	1.13
	A125	0.74	0.64	-0.15	0.63	0.05	0.82	0.86	0.25	2.96
	A129	0.18	0.22	-0.59	0.37	2.58	0.11	0.56	0.27	1.14
DIK093	A5	0.13	0.11	0.40	0.51	0.63	0.43	1.50	0.55	4.04
(N=166)	A203	0.36	0.37	0.28	0.50	0.33	0.57	1.33	0.50	3.50
	A205	0.13	0.16	-0.01	0.45	0.00	0.99	0.99	0.41	2.41
	A207	0.07	0.10	0.14	0.59	0.06	0.81	1.16	0.36	3.68
	A209	0.13	0.09	0.81	0.55	2.16	0.14	2.24	0.77	6.56
	A211	0.19	0.17	0.23	0.46	0.25	0.62	1.26	0.51	3.12
DIK2117	A5	0.12	0.09	0.42	0.49	0.71	0.40	1.52	0.58	3.99
(N=168)	A223	0.07	0.11	-0.27	0.55	0.24	0.63	0.77	0.26	2.25
	A227	0.34	0.33	0.28	0.52	0.29	0.59	1.32	0.48	3.64
	A229	0.21	0.32	-0.30	0.54	0.31	0.58	0.74	0.26	2.14
	A233	0.08	0.04	1.22	0.74	2.70	0.10	3.40	0.79	14.63
	A235	0.18	0.12	0.63	0.51	1.53	0.22	1.89	0.69	5.15
DIK2200	A5	0.15	0.10	0.43	0.36	1.46	0.23	1.54	0.77	3.09
(N=172)	A167	0.85	0.90	0.00	-	-	-	-	-	-
DIK2333	A5	0.10	0.10	0.09	0.50	0.03	0.86	1.09	0.41	2.89
(N=172)	A197	0.19	0.22	-0.01	0.41	0.00	0.98	0.99	0.44	2.22
	A198	0.10	0.08	0.18	0.47	0.15	0.70	1.20	0.48	3.02
	A199	0.14	0.17	-0.11	0.44	0.06	0.80	0.90	0.38	2.14
	A202	0.47	0.42	0.26	0.51	0.27	0.61	1.30	0.48	3.51
DIK4009	A200	0.11	0.08	0.34	0.41	0.69	0.41	1.41	0.63	3.14
(N=170)	A202	0.75	0.82	-0.91	0.92	0.97	0.32	0.40	0.07	2.46
	A204	0.14	0.10	0.23	0.45	0.26	0.61	1.26	0.52	3.04
DIK4118	A5	0.08	0.09	0.60	0.50	1.44	0.23	1.82	0.69	4.83
(N=170)	A248	0.27	0.31	0.74	0.50	2.20	0.14	2.10	0.79	5.60
	A250	0.24	0.23	1.07	0.50	4.52	0.03	2.91	1.09	7.78
	A252	0.12	0.10	1.06	0.55	3.80	0.05	2.90	1.00	8.43
	A254	0.11	0.11	0.72	0.49	2.09	0.15	2.05	0.78	5.40
	A256	0.18	0.16	1.04	0.56	3.47	0.06	2.83	0.95	8.47

Appendix III. CWD case-control association test results by conditional regression for 42 microsatellite markers in mule deer.

Marker	Allele†	Freq. case	Freq. control	Para-meter	S.E.	χ^2	*P*	Odds ratio	95% CI Lower	95% CI Upper
DIK4158	A5	0.14	0.14	-0.20	0.51	0.16	0.69	0.82	0.30	2.22
(N=170)	A233	0.51	0.54	0.04	0.53	0.01	0.94	1.04	0.37	2.93
	A261	0.11	0.13	-0.04	0.51	0.01	0.94	0.96	0.35	2.62
	A263	0.11	0.09	0.24	0.46	0.27	0.60	1.27	0.52	3.12
	A269	0.14	0.11	0.34	0.44	0.59	0.44	1.40	0.59	3.29
DIK4242	A5	0.10	0.08	-0.24	0.62	0.15	0.70	0.79	0.23	2.64
(N=166)	A278	0.25	0.23	-0.63	0.60	1.12	0.29	0.53	0.16	1.71
	A280	0.07	0.11	-0.95	0.63	2.27	0.13	0.39	0.11	1.33
	A284	0.19	0.16	-0.37	0.51	0.54	0.46	0.69	0.26	1.86
	A290	0.11	0.07	-0.27	0.69	0.16	0.69	0.76	0.20	2.91
	A292	0.11	0.10	-0.31	0.61	0.26	0.61	0.73	0.22	2.41
	A294	0.11	0.16	-0.69	0.58	1.44	0.23	0.50	0.16	1.55
	A298	0.06	0.09	-1.06	0.72	2.12	0.15	0.35	0.08	1.44
DIK4384	A5	0.07	0.06	0.66	0.63	1.08	0.30	1.93	0.56	6.65
(N=174)	A230	0.06	0.05	0.33	0.68	0.23	0.63	1.39	0.36	5.30
	A232	0.08	0.04	0.87	0.63	1.91	0.17	2.38	0.70	8.12
	A233	0.14	0.18	-0.48	0.50	0.94	0.33	0.62	0.23	1.64
	A234	0.38	0.40	-0.14	0.50	0.07	0.78	0.87	0.33	2.32
	A235	0.09	0.10	-0.08	0.54	0.02	0.88	0.92	0.32	2.67
	A236	0.17	0.18	-0.03	0.45	0.01	0.94	0.97	0.40	2.35
DIK4415	A5	0.04	0.05	-0.23	0.57	0.16	0.69	0.80	0.26	2.43
(N=168)	A208	0.49	0.40	0.38	0.34	1.21	0.27	1.46	0.75	2.84
	A210	0.48	0.55	-0.20	0.38	0.28	0.60	0.82	0.39	1.73
DIK4520	A5	0.10	0.10	-0.43	0.57	0.56	0.45	0.65	0.22	1.99
(N=168)	A219	0.07	0.10	-0.56	0.57	0.95	0.33	0.57	0.19	1.75
	A221	0.21	0.15	0.13	0.47	0.07	0.79	1.14	0.45	2.87
	A223	0.41	0.44	-0.63	0.56	1.23	0.27	0.54	0.18	1.62
	A225	0.14	0.12	-0.02	0.52	0.00	0.98	0.98	0.36	2.73
	A235	0.07	0.10	-1.00	0.68	2.18	0.14	0.37	0.10	1.39
DIK4665	A5	0.08	0.15	-0.46	0.43	1.15	0.28	0.63	0.27	1.47
(N=172)	A208	0.15	0.12	0.50	0.47	1.16	0.28	1.66	0.66	4.13
	A210	0.13	0.13	-0.02	0.50	0.00	0.97	0.98	0.37	2.61
	A214	0.15	0.12	0.29	0.43	0.45	0.50	1.33	0.58	3.09
	A216	0.49	0.48	0.28	0.50	0.30	0.58	1.32	0.49	3.52
DIK5018	A5	0.09	0.13	-0.16	0.48	0.11	0.74	0.85	0.33	2.19
(N=172)	A230	0.16	0.17	-0.11	0.40	0.07	0.78	0.90	0.41	1.97
	A232	0.45	0.35	0.48	0.47	1.03	0.31	1.62	0.64	4.09
	A240	0.30	0.34	-0.15	0.40	0.13	0.72	0.87	0.39	1.90
DIK5112	A5	0.10	0.08	-0.06	0.46	0.02	0.90	0.95	0.39	2.31
(N=166)	A205	0.07	0.06	0.14	0.50	0.07	0.79	1.15	0.43	3.09
	A207	0.72	0.75	-0.31	0.69	0.20	0.66	0.74	0.19	2.83
	A215	0.11	0.11	0.10	0.41	0.06	0.81	1.10	0.49	2.46
DIK5136*	A5	0.11	0.11	0.43	0.61	0.50	0.48	1.54	0.47	5.09
(N=168)	A264	0.11	0.20	-0.42	0.54	0.61	0.44	0.66	0.23	1.88
	A266	0.15	0.21	-0.16	0.53	0.09	0.77	0.86	0.30	2.43
	A268	0.11	0.07	0.44	0.56	0.61	0.44	1.55	0.51	4.69
	A270	0.30	0.13	1.15	0.50	5.40	0.02	3.17	1.20	8.41
	A272	0.07	0.06	0.23	0.67	0.12	0.73	1.26	0.34	4.66
	A286	0.17	0.21	-0.13	0.46	0.08	0.78	0.88	0.35	2.18
DIK5145	A5	0.09	0.09	-0.80	0.57	1.99	0.16	0.45	0.15	1.37
(N=166)	A184	0.22	0.17	-0.41	0.50	0.69	0.41	0.66	0.25	1.75
	A186	0.22	0.27	-1.17	0.50	5.50	0.02	0.31	0.12	0.83

Appendix III. CWD case-control association test results by conditional regression for 42 microsatellite markers in mule deer.

Marker	Allele†	Freq. case	Freq. control	Para-meter	S.E.	χ^2	*P*	Odds ratio	95% CI Lower	95% CI Upper
	A188	0.31	0.34	-0.89	0.59	2.29	0.13	0.41	0.13	1.30
	A190	0.16	0.13	-0.40	0.51	0.62	0.43	0.67	0.25	1.81
DIK643	A5	0.24	0.26	0.04	0.37	0.01	0.92	1.04	0.50	2.14
(N=172)	A185	0.34	0.33	0.27	0.37	0.54	0.46	1.31	0.63	2.71
	A187	0.41	0.41	0.11	0.38	0.08	0.77	1.12	0.53	2.35
FCB48	A5	0.13	0.15	-0.19	0.36	0.29	0.59	0.82	0.41	1.67
(N=172)	A171	0.87	0.85	-0.19	0.89	0.05	0.83	0.82	0.14	4.74
HUJ616	A125	0.06	0.05	0.81	0.66	1.50	0.22	2.24	0.62	8.15
(N=168)	A129	0.13	0.08	1.02	0.55	3.46	0.06	2.77	0.95	8.12
	A135	0.58	0.61	0.71	0.63	1.27	0.26	2.03	0.59	6.95
	A137	0.05	0.07	-0.10	0.52	0.04	0.84	0.90	0.33	2.50
	A139	0.17	0.20	0.06	0.39	0.02	0.88	1.06	0.50	2.28
ILSTS059	A5	0.31	0.30	0.31	0.44	0.51	0.48	1.37	0.58	3.21
(N=168)	A169	0.36	0.34	0.33	0.38	0.73	0.39	1.38	0.66	2.92
	A175	0.33	0.36	0.37	0.41	0.78	0.38	1.44	0.64	3.25
ILSTS086	A5	0.18	0.17	0.68	0.57	1.41	0.24	1.97	0.64	6.02
(N=170)	A183	0.06	0.06	0.37	0.62	0.36	0.55	1.45	0.43	4.85
	A187	0.34	0.31	0.82	0.57	2.05	0.15	2.27	0.74	6.95
	A189	0.11	0.13	0.42	0.56	0.56	0.45	1.52	0.51	4.52
	A191	0.11	0.11	0.48	0.53	0.82	0.37	1.62	0.57	4.57
	A197	0.06	0.10	-0.26	0.59	0.20	0.65	0.77	0.24	2.45
	A199	0.08	0.06	0.72	0.55	1.70	0.19	2.05	0.70	6.03
	A201	0.06	0.06	0.28	0.62	0.21	0.65	1.33	0.39	4.48
INRA111	A5	0.06	0.10	-0.70	0.54	1.65	0.20	0.50	0.17	1.44
(N=172)	A129	0.06	0.10	-0.65	0.49	1.74	0.19	0.52	0.20	1.37
	A135	0.07	0.07	-0.20	0.51	0.16	0.69	0.82	0.30	2.22
	A137	0.52	0.46	-0.20	0.49	0.17	0.68	0.82	0.31	2.15
	A139	0.11	0.17	-0.80	0.47	2.95	0.09	0.45	0.18	1.12
	A141	0.17	0.10	0.48	0.47	1.07	0.30	1.62	0.65	4.03
INRA177	A5	0.21	0.18	0.31	0.37	0.72	0.40	1.37	0.66	2.84
(N=168)	A93	0.49	0.58	0.15	0.55	0.07	0.78	1.16	0.39	3.45
	A101	0.15	0.14	0.25	0.47	0.28	0.59	1.28	0.51	3.22
	A113	0.15	0.10	0.67	0.45	2.24	0.13	1.95	0.81	4.68
MNS-97	A275	0.39	0.38	-0.04	0.38	0.01	0.92	0.96	0.46	2.03
(N=168)	A277	0.61	0.62	-0.11	0.46	0.05	0.82	0.90	0.36	2.22
UMBTL184	A5	0.08	0.02	1.40	0.71	3.91	0.05	4.06	1.01	16.30
(N=166)	A275	0.28	0.33	-0.14	0.47	0.10	0.76	0.87	0.35	2.16
	A277	0.10	0.13	-0.17	0.52	0.11	0.74	0.84	0.31	2.32
	A281	0.08	0.12	-0.38	0.50	0.57	0.45	0.69	0.26	1.82
	A283	0.19	0.21	-0.23	0.52	0.20	0.66	0.80	0.29	2.19
	A285	0.27	0.18	0.51	0.52	0.98	0.32	1.67	0.61	4.62
UMBTL70	A5	0.11	0.07	1.11	0.56	3.94	0.05	3.02	1.01	9.00
(N=168)	A177	0.23	0.33	0.10	0.44	0.05	0.83	1.10	0.46	2.63
	A189	0.10	0.10	0.78	0.59	1.75	0.19	2.19	0.69	6.96
	A193	0.16	0.13	0.69	0.47	2.14	0.14	1.99	0.79	5.02
	A195	0.29	0.18	1.16	0.54	4.61	0.03	3.19	1.11	9.18
	A197	0.07	0.09	-0.07	0.58	0.01	0.91	0.93	0.30	2.93
	A201	0.05	0.10	0.18	0.60	0.09	0.76	1.20	0.37	3.88
URB021B	A5	0.07	0.08	-0.08	0.51	0.02	0.88	0.92	0.34	2.53
(N=168)	A145	0.65	0.63	0.93	0.57	2.67	0.10	2.54	0.83	7.75

Appendix III. CWD case-control association test results by conditional regression for 42 microsatellite markers in mule deer.

Marker	Allele†	Freq. case	Freq. control	Para-meter	S.E.	χ^2	*P*	Odds ratio	95% CI Lower	95% CI Upper
	A153	0.15	0.20	0.15	0.39	0.14	0.71	1.16	0.54	2.48
	A155	0.13	0.09	0.61	0.44	1.94	0.16	1.85	0.78	4.38

Maximum likelihood estimation of conditional logistic regression parameters and odds ratios were calculated in SAS v9.2. Chi-square (df = 1) and CI were calculated using Wald statistics.

* Indicates markers that were significant in global tests ($\alpha = 0.05$).

† Pooled rare alleles (< 0.05) are denoted as A5.