AN EVALUATION OF THE OUTCOME OF INTERSPECIFIC HYBRIDIZATION EVENTS COINCIDENT WITH A DRAMATIC DEMOGRAPHIC DECLINE IN NORTH AMERICAN BISON

A Dissertation

by

TODD J. WARD

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2000

Major Subject: Genetics

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ABSTRACT

An Evaluation of the Outcome of Interspecific Hybridization Events Coincident with a Dramatic Demographic Decline in North American Bison. (May 2000)

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Chair of Advisory Committee: Dr. James N. Derr

Process oriented studies of hybridization have largely been aimed at gaining insights into the nature of species barriers, through an examination of introgression in natural hybrid zones. However, the debate over the evolutionary significance of hybridization has gained renewed interest due to the recognition that hybridization is often a secondary consequence of habitat modification and population fragmentation that may pose an additional threat to conservation dependent populations. Studies of natural hybrid zones often lack the historical and biological information required to test alternative hypotheses regarding the evolutionary significance of introgressive hybridization, and may not provide an appropriate model for examining the dynamics of introgression in conservation dependent populations. However, historical hybridization between North American bison and domestic cattle is well documented, and took place at the peak of a dramatic decline in North American bison populations. A characterization of the outcomes of these historical hybridization events allows for an explicit test of the role of introgressive hybridization in evolution, and an examination of the dynamics of introgressive hybridization in conservation dependent populations. Therefore, a species specific mtDNA marker was used to identify domestic cattle introgression in seven of 16 North American bison populations examined. The extent of nuclear introgression in North American bison populations was assessed using a set of 20 diagnostic microsatellite markers on 12 of the 29 autosomes, and diagnostic markers on the X and Y chromosomes. Taking advantage of linkage disequillibrium created in admixed populations, sets of closely linked microsatellite markers were used to identify the

introgression of domestic cattle chromosomal segments in seven different regions of the genome. Contrary to predictions based on a view of hybridization as a creative evolutionary force, observed levels of introgression were consistent with expectations generated via simulation using a model that was neutral with respect to exogenous selection, indicating that domestic cattle variation had not been selectively maintained in the North American bison genome. However, simulation results also indicated that hybridization could slightly reduce the probability of population extinction and the loss of native genetic variation, even when there is strong endogenous selection against hybrid individuals.

This dissertation is dedicated to my wife, Holly, and my children, Hannah, Charles, and Abbigail. Their love, support, and sacrifice made it possible for me to complete this work.

ACKNOWLEDGMENTS

First, I would like to thank my committee chair, Dr. James Derr, for providing the resources and encouragement that I needed to complete my dissertation work. Special thanks go to Dr. Scott Davis, Dr. Rodney Honeycutt, and Dr. Joe Templeton for serving on my advisory committee and for providing me with support and guidance throughout my time at Texas A&M. I am greatly indebted to Dr. Ellen Williamson, whose programming skills and advice made the simulation analysis possible, and to Mr. Robert Schnabel for extensive discussion of the material in this manuscript and for considerable help in the laboratory. I would also like to thank Dr. Loren Skow, Dr. Dan Gallagher, and Mr. Chad Nall for contributing information on the Y-specific microsatellite marker, Dr. Dan Bradley for providing mtDNA sequence data for domestic cattle, and Dr. Jim Womack for providing many of the fluorescently labeled microsatellite primers used in this study. Thanks go to Dr. Heather Streppa, Ms. Natalie Rayford, and Ms. Claire Kolenda for their help in the laboratory. I would like to thank the Texas Parks and Wildlife Department for providing some of the funding for this research, and Mr. Danny Swepston for providing information on the history of the Caprock Canyon State Park bison population. Thanks also go to Mr. Kevin Sherman, Mr. David Wiseman, Mr. Tom Norman, Mr. Ron Walker, and to Ms. Barbara Muenchau for providing essential information on various bison populations. Finally, I would like to thank Dr. Joe Bielawski and Dr. Alex Rooney for helpful discussions of this research and evolutionary genetics in general.

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INTRODUCTION

The Evolutionary History of Cattle and Bison

The Bovini is one of three bovine tribes that first appeared during the Miocene, when major radiations of many groups of higher ruminants were occurring in response to an increasing availability of herbaceous low growing vegetation (McDonald 1981). The major Bovini radiation occurred during the Pliocene of southeastern Asia, with all modern genera appearing in the late Pliocene to early Pleistocene (Pilgrim 1947; Gentry 1978; McDonald 1981). Most authors recognize four extant Bovini genera: *Syncerus* and *Bubalus*, the true buffaloes of Africa and Asia respectively; *Bos*, domestic cattle and their wild relatives; and *Bison*, the North American and European bison (Simpson 1984; Nowak 1999). There is some disagreement over the phylogenetic relationships among the cattle and bison, in that *Bison* has been syonymized with *Bos* in some classifications (Van Gelder 1977; Gentry 1978; Miyamoto *et al.* 1989). Despite this uncertainty regarding the monophyly of *Bos* and *Bison*, there is a general consensus that *Bos* and *Bison* represent a single monophyletic clade derived from a common ancestor roughly two million years ago (McDonald 1981; Janecek *et al.* 1996).

The genus *Bos* is known from the Pleistocene of Asia and Europe with rare advances into North America (McDonald 1981). Extant cattle species include *Bos gaurus* (gaur), *Bos javanicus* (banteng), and *Bos sauveli* (kouprey) which all have tropical or sub-tropical distributions near the center of the Bovini radiation (National Research Council 1983; Nowak 1999). In contrast, *Bos grunniens* (yak) is adapted to desolate mountain areas where it lives at altitudes of 4,000 to 6,000 meters (National Research Council 1983). Of course, the two species of domestic cattle (*Bos taurus* and *Bos indicus*) are the most well known, widely distributed, and economically important members of this genus. These two domestic species were derived from a wild progenitor (*Bos primigenius*) that successfully expanded its range to include much of Europe and Asia before human induced habitat alterations led to its eventual extinction in the seventeenth century (Macdonald 1984).

Traditionally, *B. taurus* (taurine cattle) and *B. indicus* (zebu cattle) have been viewed as subspecies, recently domesticated from the same wild stock (Epstein 1971; Epstein and Mason 1984; Payne 1991; Nowak 1999). However, recent investigations of

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This dissertation follows the style and format of the journal Genetics.

genetic differentiation at mitochondrial and microsatellite loci indicate that taurine and zebu cattle were domesticated independently from separate stocks of *B. primigenius* at least 200,000 years ago (Loftus *et al.* 1994; MacHugh *et al.* 1997). These same investigations also indicated that all African cattle breeds have taurine mitochondrial haplotypes, and that breeds of African zebu cattle resulted from male mediated introgression of genes from Indian zebu breeds.

Bison remains have been identified at least as far back as the late Pliocene of central Asia (Ranov and Davis 1979), and are known from the Pleistocene of Asia, Europe, and North America (McDonald 1981). However, prior to the Donau glaciation, about 1.5 million years ago, *Bison* were confined to temperate and upland east Asia (McDonald 1981), and Guthrie (1970) hypothesized that they may have been competitively excluded from southward expansion by *Bos*. Similarly, *Bison* may have served as a biotic barrier to the northward expansion of *Bos* (Guthrie 1970), with the exception of an ephemeral expansion of yak, or a closely related form, into Alaska (Frick 1937).

The genus *Bison* is represented by two extant species, *Bison bison* (North American bison) and *Bison bonasus* (European bison). The evolutionary and biogeographical histories of these species have been the subject of considerable debate and remain largely unclear (Allen 1876; Schultz and Frankforter 1946; Skinner and Kaisen 1947; Fuller and Bayrock 1965; Guthrie 1970; Geist 1971; McDonald 1981). However, it appears that at an early stage during the Illinoian glaciation, 400,000 to 500,000 years ago, *Bison* spread across northern Eurasia and into Alaska (Guthrie 1970; McDonald 1981). Unimpeded by competition from *Bos*, *Bison* spread not only eastward across North America but also as far south as El Salvador (Macdonald 1984), thus becoming the only genus in the tribe Bovini to achieve a lasting presence in the New World.

Evolution and Taxonomy of North American Bison

Three North American (*B. antiquus*, *B. latifrons*, and *B. bison*), and two European (*B. alaskensis* and *B. priscus*) autochthonous species of *Bison* are believed to have existed in North America (McDonald 1981). However, *B. bison* (North American bison) is the only extant species of *Bison* in North America. The precise timing and location for the origin of North American bison is unclear, but it is generally believed that they evolved in mid-latitude North America sometime after the Wisconsin glaciation, about 10,000 years ago (McDonald 1981). As the distribution of North American bison grew to

its primary and historic range, about 3,000 years ago, it became the dominant large herbivore in North America (McDonald 1981).

The taxonomy of North America bison has traditionally included recognition of two distinct subspecies, *B. bison athabascae* (wood bison) and *B. bison bison* (plains bison), on the basis of demonstrable character clines extending north to south (Rhoads 1897). However, considerable disagreement exists as to whether this character cline is the result of genotypic or ecotypic differences between various populations (McDonald 1981: Geist 1991; van Zyll de Jong *et al.* 1995). Characterization of the historical subdivision between wood and plains bison populations was greatly complicated by introductions, from 1925 to 1928, of more than 6,000 plains bison into the last wood bison population. In addition to the distinctions made between wood and plains bison, a further distinction is often made between plains bison from the southern plains and those from the northern plains, although this distinction has not been formalized.

Population Decline and Recovery

At the end of the nineteenth century, a highly publicized and well documented demographic decline in North American bison led to the near extinction of the species and left the remaining wild animals in small fragmented groups scattered across their former range. Prior to European settlement, the number of North American bison was estimated to be in the tens of millions (Seton 1929), but by the turn of the century, only 634 were left in the wild (Hornaday 1913). Flores (1991) provides some evidence that North American bison populations had been declining since the seventeenth century because of increased hunting pressure from native Americans, precipitated by the introduction of the domestic horse, and by widespread epidemics of diseases introduced by domestic cattle. However, the near extermination of the North American bison was ultimately the result of overhunting by Europeans supplying the robe trade (Roe 1951), and was encouraged by government agencies in the U.S., which hoped that the disappearance of the North American bison would help them gain control over native Americans who depended on the North American bison for their survival (Coder 1975). In Hornaday's (1913) description of the rapid decline of North American bison, he indicates that the completion of the Union Pacific Railway in 1869 facilitated the slaughter of bison on the southern plains, which he says was complete by 1875, leaving only "three very small bunches" of southern plains bison in the wild. He also indicates that the completion of the Northern Pacific Railway in

1880 led to a "grand attack" upon the northern herd, which resulted in the destruction of all but about 800 animals by 1883.

The decline in North American bison numbers ended around 1888, when there were an estimated 541 North American bison in existence (Coder 1975). Efforts to save North American bison from extinction were independently undertaken by a small number of individuals throughout the U.S. and Canada, who each captured a few of the remaining North American bison from the wild, and raised them in captivity. Five of these populations are recognized as playing a major role in the recovery of North American bison. These include the McKay-Alloway population from Saskatchewan, the Pablo-Allard population from Montana, the Dupree population from Montana, the Goodnight population from the Texas panhandle, and the Jones population from Kansas, Nebraska, and Texas. The numbers of founders, and the early histories of each of these five "foundation" herds is reviewed in Coder (1975), but in total, these five populations consisted of no more than 90 founding individuals. Eventually, the U.S. government joined in the effort to save North American bison from extinction by protecting the wild population of plains bison in Yellowstone National Park. The Canadian government similarly acted to protect the last wild population of wood bison, forming Wood Buffalo National Park. Captive individuals were introduced into these populations, but they are the only surviving populations of North American bison to have continuously existed in the wild.

Once North American bison were afforded protection from hunters, their numbers increased rapidly. Between 1888 and 1902, the number of North American bison had more than doubled, and they were considered safe from extinction by 1909 (Coder 1975). Currently there are about 20,000 North American bison in publicly managed populations throughout the U.S. and Canada (Wilson and Strobeck 1999). In addition, there are 200,000 to 300,000 North American bison in private holdings, many of which should be considered domestic animals (Yorks and Capels 1998). Manifestations of reduced reproductive potential or other deleterious consequences typically associated with bottlenecked populations have been rare, especially among the animals in publicly managed populations (Berger and Cunningham 1994). Due to this remarkable recovery, the restoration of North American bison is viewed as one of the first conservation success stories, and the North American bison has become a symbol of natural resource conservation.

History of Hybridization

One aspect of the recovery of North American bison that is often overlooked, is that in addition to an interest in saving North American bison from extinction, many of the individuals involved in the formation of the five private "foundation" herds were also interested in improving various production characteristics of their domestic cattle through hybridization with North American bison. For example, the Goodnight herd was started in 1878 (Coder 1975). From 1885 until 1910, Charles Goodnight produced hybrids between North American bison bulls and domestic cows (Polled Angus breed) in order to create an animal that possessed the hardiness of the North American bison, and the production potential of domestic cattle. A similar attempt to improve various traits of domestic cattle was made by C. J. "Buffalo" Jones, whose North American bison population was started in 1886, and was perhaps the most important of the five "foundation" herds, as it included many more founders than did the other populations (Coder 1975). Jones began his hybridization experiments in 1888 in order to "produce a race of cattle equal in hardiness to the buffalo, with robes much finer, and possessing all the advantages of the best bred cattle" (Dary 1989).

Although detailed hybridization experiments were not conducted in the McKay-Alloway, Pablo-Allard, or Dupree populations, hybrid individuals were reported in each of these populations. For example, in 1879 Charles Alloway claimed that his population contained 13 "pure" North American bison and three hybrids (Coder 1975). Although it is possible that these three hybrids were sold, it is unclear if any more hybridization events took place in this population. However, there is a large amount of information documenting a long history of hybridization in the Dupree herd. Fredrick Dupree captured five North American bison calves in 1883 and pastured them with domestic cattle (Coder 1975). By 1888, there were nine "pure" North American bison and seven hybrids in this population (Coder 1975). According to Coder (1975) this population continued to grow and cross with domestic cattle until Fredrick Dupree's death in 1898. In contrast to these previous examples, the Pablo-Allard herd is the only one of the five "foundation" herds where there is no documentation indicating that hybridization between domestic cattle and North American bison was taking place. However, Michael Pablo and Charles Allard did buy 18 hybrids from C. J. Jones in 1893 (Coder 1975). These animals were sequestered on an island in the middle of Flathead lake, and were reportedly not allowed to mix with the pure North American bison in this population (Coder 1975).

Reproductive Biology of North American Bison x Domestic Cattle Hybrids

North American bison and domestic cattle are interfertile. However, crosses between these two species result in heavy losses of offspring and dam, usually attributed to hydrops amnia, an excessive secretion of the amniotic fluid (Jones 1907; Boyd 1908; Goodnight 1914; Gray 1954). However, this condition is far less severe in crosses between North American bison females and domestic cattle males than in the reverse cross (Jones 1907; Gray 1954; Dary 1989). Regardless of the direction of the cross, first generation (F1) hybrid offspring of both sexes display reduced fertility and viability relative to either North American bison or domestic cattle. However, this reduction is most pronounced in male F1 hybrids (Jones 1907; Boyd 1908, 1914; Goodnight 1914; Gray 1954), which are completely sterile due to the absence of germinal epithelium, and are only rarely viable (Gray 1954). These findings are consistent with the observation that in cases of preferential sterility or inviability, the heterogametic sex is most affected (Haldane 1922). This observation is characteristic of speciation in all known animals (Orr 1997), and indicates that in the course of divergent evolution, domestic cattle and North American bison have accumulated mutations effecting fertility and viability that are partially recessive in a hybrid genetic background. However, the fact that female F1's are at least partially fertile, makes introgressive hybridization possible, and normal viability and fertility for both sexes is quickly regained in subsequent backcross generations produced with either domestic cattle or North American bison (Boyd 1908, 1914).

Despite the fact that losses due to hydrops amnia are reduced in crosses of domestic cattle bulls and female North American bison, historical reports indicate that the reverse cross was most common (Jones 1907; Boyd 1908, 1914; Goodnight 1914; Coder 1975; Dary 1989). For example, in the first detailed report of human induced hybridization between the two species in 1854, Robert Wickliffe reports that North American bison bulls would readily breed domestic cows, but his domestic cattle bulls were "always shy of the buffalo cow" and would not accede to attempts to get them to breed North American bison (Dary 1989). This observation is repeated in many of the accounts of hybridization (Dary 1989), including that of Boyd (1908, 1914) who conducted a detailed study of hybridization in North American bison and domestic cattle. Boyd found it impossible to cross domestic cattle bulls with North American bison females due to the wariness of the domestic cattle bull. Therefore, it appears that there may be some limited pre-zygotic reproductive barriers in the form of behavioral constraints on the direction of hybridization.

The Significance of Introgressive Hybridization in Evolution

Hybridization between previously isolated and differently adapted species or populations has been viewed by many researchers as a potentially creative evolutionary force, even in cases where early generation hybrids display reduced fitness relative to either parental group (Lotsy 1931; Anderson 1948; Anderson and Stebbins 1954; Klier *et al.* 1991; Arnold 1997). This adaptationist view of hybridization has predominated among botanists, presumably because of the large number of perceived cases of hybridization between plant taxa (Riesberg and Wendel 1993), and the apparently large role of polyploid speciation in the diversification of the angiosperms (Arnold 1997). Obviously, the generation of novel evolutionary lineages via hybridization, which are reproductively isolated from their parental taxa, is pertinent to discussions of the overall evolutionary importance of hybridization. However, the focus here will be limited to the evolutionary significance of introgressive hybridization.

The term, introgressive hybridization, was first used by Anderson and Hubricht (1938) to describe the transfer of genes between differentiated populations or species via the production of fertile hybrids capable of backcrossing with one or both parental taxa. Anderson (1948) argued that the chief result of introgressive hybridization would be the enrichment of variation in the participating species. Anderson and Stebbins (1954) added that introgressive hybridization allowed for the transfer of novel adaptations, and that hybridization between species may generate new adaptive norms, allowing for the exploitation of novel ecological niches. They also emphasized that these hybrids would demonstrate enhanced evolutionary plasticity due to the crossing over of introgressed segments, which would generate new genetic diversity more rapidly than mutation. However, these authors have recognized that hybrids often display reduced fitness, and have argued that they will only persist in new ecological niches (hybridized habitats) in which they are more fit than either parental species (Anderson 1948). The prevalence of hybrids in disturbed habitats is taken as evidence for this requirement. Arnold (1997) argues against this viewpoint, rejecting the notion that hybrids are necessarily less fit than their progenitors, and advances the idea that the prevalence of hybrids in disturbed areas is the result of greater opportunity for hybridization to occur in these areas because of the breakdown of ecological barriers.

The argument that introgressive hybridization leads to increased genetic diversity was further advanced by Rattenbury (1962), who introduced the idea of cyclic hybridization. He hypothesized that natural selection would favor species that through

dispersal, exist as semi-isolated populations. These populations could differentiate through drift in isolation, with rare instances of contact and hybridization providing for large influxes of variation from different and often ecologically or geographically segregated groups. Thus, there could be a continuing fluctuation in allele frequencies that would correspond with fluctuations in environmental conditions.

Of course, the view of introgressive hybridization as a potentially creative evolutionary force has not been limited to botanists. For example, Lewontin and Birch (1966) demonstrated that hybridization between two species in the genus *Dacus* could have provided the genetic variation necessary to create a new adaptive norm, resulting in an expanded ecological tolerance and geographic range. They conducted controlled laboratory experiments, based on an actual range expansion by species of *Dacus* which regularly exchanged genes via introgressive hybridization, but which had maintained their specific identity because of selection against hybrid individuals. Their results indicated that the introduction of genes from one species into another could have provided for adaptive evolution even in cases where hybrids are less fit than their parental species in their own environment, and when the introgressed genes are not themselves necessarily adaptive. A study of hybridization in ground finches from the Galapagos by Grant and Grant (1992) similarly found that introgressive hybridization made rapid shifts between adaptive peaks possible, which allowed the participating species to effectively track fluctuating ecological conditions, even though hybrids may have been at a selective disadvantage in some environments. They concluded that hybridization was evolutionarily significant because it generated novel combinations of genes, creating favorable genetic conditions for rapid and major evolutionary change.

In contrast to a view of hybridization as a creative evolutionary force, many zoologist have considered hybridization to be an evolutionary dead end. This view has been attributed to the perception that interspecific hybridization among animals is rare and maladaptive (Harrison 1993; Rhymer and Simberloff 1996; Dowling and Secor 1997). This view of hybridization goes back at least as far as Darwin (1859), but has intensified during the period since the modern synthesis. Dobzhansky (1951) argued that the frequency of successful hybridization in animals would be reduced by their greater complexity, requiring that the adaptive value of genotypes would depend upon the integration of larger numbers of genes than would be required by plants. Mayr (1963) similarly argued that hybridization was decidedly maladaptive because it caused the disruption of co-adapted gene complexes. However, while Dobzhansky and Mayr did not

view cases of hybridization as evolutionarily important in there own right, they recognized that such cases were potential opportunities to study the processes of genetic divergence and speciation (Dobzhansky 1937, 1940; Mayr 1942). Of course, the notion that hybridization was necessarily maladaptive served as the theoretical framework for these investigations.

The process oriented approach to studies of hybridization, initiated by Mayr and Dobzhansky, has gained renewed attention over the last several decades in the form of detailed studies of hybrid zone dynamics (Harrison 1993; Arnold 1997). Common views of the nature of hybrid zones include the idea that they are maintained by selection along environmental gradients (Endler 1977) and that they are characterized by an ecotone in which hybrids are more fit than either of the distinct and relatively uniform parental populations on either side of the zone (Moore 1977). However, the theoretical framework for the majority of hybrid zone studies is the view that hybrid zones are maintained in a balance between dispersal and selection against hybrids independent of the environment, as described by Barton and Hewitt (1985). This framework is obviously related to that used by Mayr (1942) in his negative assessment of the role of hybridization in evolution and in the delineation of the biological species concept. While this philosophical approach has certainly been beneficial for examining processes related to speciation, it has also limited process oriented examinations of hybridization itself, including the potential role of hybridization in the maintenance of genetic diversity and adaptive evolution. In addition, studies of naturally occurring hybrid zones usually lack the biological and historical information required to generate explicit null models and neutral expectations. Differential introgression of marker loci can be used to indicate positive selection, however without the capacity to falsify the null hypothesis of neutral introgression, this can not be proven (Clark 1985; Arnold 1997).

Implications of Hybridization for Conservation Biology

Widespread habitat alteration, population fragmentation, and the introduction of exotic and domestic species into areas inhabited by closely related species can greatly increase the potential for hybridization to occur (Lehman *et al.* 1991; Boyd and Houpt 1994; Karl *et al.* 1995). The increasing number of species threatened by habitat alteration and population fragmentation have therefore generated greater interest in the possible outcomes of introgressive hybridization for conservation dependent populations or species (Avise 1994; Rhymer and Simberloff 1996; Arnold 1997). Differences in opinion about

the impact of introgressive hybridization on conservation dependent populations are predictably connected to views about the adaptive value of introgression and hybridization. When a species that is already rare comes into contact with a more numerous species, outbreeding depression and genetic assimilation may occur (Cade 1983; Fritsch and Riesberg 1996). The degree to which this is generally of concern to conservation biologists is dependent on the extent to which hybrids are seen as possessing reduced fitness. For instance, concerns over outbreeding depression and the loss of reproductive effort (Ellstrand and Elam 1993) could be countered with the argument that introgressive hybridization could result in the introduction of critically needed genetic variation, increasing evolutionary plasticity and allowing the rare species to adapt to environmental changes (Rhymer and Simberloff 1996; Dowling and Secor 1997). Additionally, rare taxa could benefit from hybridization by acquiring novel adaptations, and hybrids could increase effective population sizes, reducing the effects of genetic drift and inbreeding depression. Arnold (1997) has characterized this possibility in terms of hybrid individuals acting as an "ark" in which native genetic variation is retained. The consideration of cases of introgression involving rare taxa will therefore require an understanding of the dynamics of introgression in small populations, and an assessment of the impact of hybridization on population fitness.

Study Rationale and Objectives

The well documented hybridization that took place between North American bison and domestic cattle at the end of the last century raises questions about the extent of introgression in current populations and the impact that hybridization had on the recovery of North American bison from near extinction. In addition to a large amount of historical information detailing the rate and relative success of hybridization in "founding" populations of North American bison, a great deal of genetic information, including a well defined linkage map, is available for domestic cattle. The availability of such detailed historical and biological information provides a nearly unique opportunity to examine the outcome of introgressive hybridization, and to test the predictions of alternative theories regarding the role of introgressive hybridization in evolution. Therefore, this study uses mitochondrial and nuclear markers to identify contemporary North American bison populations with a hybrid ancestry. The frequency and genomic distribution of introgressed material in these populations is compared with expectations derived from a simulation model based on a known population history and the assumption of selective

neutrality, in order to test for the effects of environment dependent (exogenous) selection. Thus, the significance of hybridization for the recovery of North American bison and for their future management can be assessed. In addition, an explicit test of the role of exogenous selection in hybrid populations can be conducted. The fact that hybridization events were coincident with a dramatic population decline in North American bison also allows for an examination of the dynamics of introgression in small or bottlenecked populations.

IDENTIFICATION OF DOMESTIC CATTLE MITOCHONDRIAL INTROGRESSION IN WILD CATTLE AND BISON SPECIES*

Synopsis

Many species are currently undergoing reductions in population size due to widespread habitat loss and expanding human activities. Because interspecific hybridization is often a consequence of population decline and fragmentation, identification of individuals or populations with hybrid ancestry is an increasingly important issue in conservation biology. In many wild cattle and bison species, the problem of natural hybridization has been compounded by indiscriminate crossbreeding with domestic cattle for the purpose of improving domesticated stocks. Accurate identification of wild cattle and bison populations with a hybrid ancestry is the first step in determining the impact that hybridization has had on these species, and is required in order to make appropriate decisions regarding their future management. Therefore, a genetic test using the polymerase chain reaction was developed so that wild cattle and bison with domestic cattle mitochondrial DNA (mtDNA) haplotypes could be rapidly identified. Using this genetic test, domestic cattle mtDNA haplotypes were detected in *Bos grunniens* (yak), *Bison bonasus* (European bison), and seven of the 16 (43.8%) *Bison bison* (North American bison) populations tested. In total, 36 of 610 (5.9%) North American bison. were found to have domestic cattle mtDNA. Haplotype data from these individuals was used in conjunction with historical records to identify three independent origins of mitochondrial introgression in North American bison.

Introduction

The recent history of *Bos* (cattle) and *Bison* (bison) has been defined by population decline and fragmentation caused by excessive hunting and human induced habitat perturbation. This led to the disappearance of *Bos primigenius* (aurochs), the ancestor of modern domestic cattle (*Bos taurus* and *Bos indicus*), from Asia and Africa about 2,000 years ago, and the eventual extinction of aurochs in Europe during the seventeenth century

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*Reprinted in part, with permission from "Identification of domestic cattle hybrids in wild cattle and bison species: a general approach using mtDNA markers and the parametric bootstrap" by T. J. Ward, J. P. Bielawski, S. K. Davis, J. W. Templeton, and J. N. Derr, 1999. *Animal Conservation*, 2, 51-57. Copyright 1999 by Cambridge University Press (see Appendix D).

(Nowak 1999). In addition, the same combination of excessive hunting and habitat loss or alteration has led to massive population declines for all wild cattle and bison species. At present, *Bos gaurus* (gaur), *Bos grunniens* (yak) and *Bos sauveli* (kouprey) are all listed in appendix 1 of the Convention on the International Trade of Endangered Species (CITES) (Nowak 1999). In addition, *Bos javanicus* (banteng) and *Bison bonasus* (European bison) are listed as endangered by the International Union for Conservation of Nature (IUCN) (Nowak 1999). While there are approximately 20,000 *Bison bison* (North American bison) in publicly managed populations (Wilson and Strobeck 1999), they have recently undergone a major bottleneck event, and are considered a conservation dependent species by the IUCN (Nowak 1999), with *B. bison athabascae* (wood bison) listed in appendix 2 of the CITES (Nowak 1999).

In addition to habitat loss and reductions in population size, many wild cattle and bison species are further threatened by hybridization with domestic cattle, with which all wild cattle and bison species can produce fertile hybrids (Nowak 1999). Historically, the practice of hybridization with wild species has been used in efforts to improve the genetic characteristics of domesticated stock. For example, banteng were crossed to domestic cattle on the island of Madura nearly 1500 years ago and their descendants are currently distributed across Indonesia (National Research Council 1983). Hybridization between yaks and domestic cattle has taken place throughout Asia, and Schaller and Wulin (1996) report hybrids in herds of wild yak from the Chang Tang Reserve of the Tibet Autonomous Region. Furthermore, North American bison were crossed with domestic cattle more than 100 years ago (Jones 1907; Boyd 1914; Goodnight 1914), which may have resulted in the introgression of domestic cattle genes into bison populations that contributed to the founding stocks of some contemporary bison herds. For instance, in a limited survey based on restriction fragment length polymorphism (RFLP) data from the mitochondrial control region, Polziehn *et al.* (1995) identified two North American bison with domestic cattle mtDNA haplotypes in the Custer State Park population

The problem of interspecific hybridization is not restricted to the wild relatives of domestic cattle. Widespread habitat alteration, population fragmentation, and the introduction of exotic or domestic species into areas inhabited by closely related species have resulted in greater opportunities for interspecific hybridization (Rhymer and Simberloff 1996). Examples include the coyote and gray wolf (Lehman *et al.* 1991), Przewalski's horse and domestic horse (Boyd and Houpt 1994), and various combinations of Kemp's ridley, loggerhead, hawksbill, and green turtles (Karl *et al.* 1995).

For endemic or threatened species, the negative consequences of interspecific hybridization can be both biological and legal. Interspecific hybridization can result in the loss of native genetic variation (genetic swamping) and the disruption of locally adapted gene complexes (outbreeding depression), potentially exacerbating other problems faced by endemic or threatened species (Avise 1994). In addition, the presence of hybrid animals in remaining populations of threatened species may result in legal challenges to their protected status (O'Brien and Mayr 1991; Hill 1993; Rhymer and Simberloff 1996). However, proponents of a view of hybridization as a creative evolutionary force argue that hybridization could be beneficial for previously isolated or threatened species (Rhymer and Simberloff 1996; Arnold 1997). For instance, introgressive hybridization could allow for the introduction of much needed genetic variation, the transfer of adaptive traits, or the retention of native genetic variation during bottleneck episodes (Rhymer and Simberloff 1996; Arnold 1997; Dowling and Secor 1997).

Evaluating the impact that hybridization has had on endemic or threatened populations, and integrating consideration of hybridization into plans for future species management, requires that populations and individuals with a hybrid ancestry be identified. Because of their rapid rate of substitution and clonal pattern of inheritance, mitochondrial markers have been effectively used to differentiate between closely related species and identify interspecific hybridization (Carr *et al.* 1986; Spolsky and Uzzell 1986; Lehman *et al.* 1991; Wayne and Jenks 1991; Painter *et al.* 1993; Avise *et al.* 1997; Mukai *et al.* 1997). Therefore, a simple genetic test, capable of rapidly identifying domestic cattle mtDNA haplotypes, was developed using nucleotide sequence data from the mitochondrial control region of yak, gaur, European bison, North American bison, and 14 breeds of domestic cattle. This genetic test was then used to determine the scope of domestic cattle introgression in a sample of 610 North American bison from 15 public populations and one privately managed population. In addition, the ability of this test to discriminate between domestic cattle haplotypes, and those of European bison, yak, and gaur was demonstrated (Table 1).

Materials and Methods

The nucleotide sequence of a 677 base-pair (bp) segment from the highly variable control region of the mitochondrial genome was determined for *B. bison* (59 animals), *B. bonasus* (3 animals), *B. grunniens* (2 animals), *B. gaurus* (1 animal), *B. taurus* (Longhorn breed, 3 animals) and *B. indicus* (Nellore breed, 1 animal) (AF083353 -

TABLE 1

Wild cattle and bison screened for domestic cattle mtDNA haplotypes

Species	Location	Sample Size
Bos gaurus	Omaha Zoo, NE	1
Bos grunniens	Brookfield Zoo, IL	1
	Kansas City Zoo, MS	1
	Sunset Zoo, KS	1
Bison bonasus (Czar's Herd)	Puschino Research Station, Russia	4
Bison bison athabascae	Elk Island National Park, Canada	19
(wood bison)	Mackenzie Bison Sanctuary, Canada	23
	Wood Buffalo National Park, Canada	23
Bison bison bison	Antelope Island State Park, UT	95
(plains bison)	Caprock Canyon State Park, TX	38
	Custer State Park, SD	34
	Elk Island National Park, Canada	25
	Finney Game Refuge, KS	26
	Fort Niobrara NWR, NE	34
	Henry Mountains, UT	21
	Maxwell Game Refuge, KS	39
	National Bison Range, MT	113
	Wind Cave National Park, SD	37
	Witchita Mountains NWR, OK	37
	Yellowstone National Park, WY	35
	Williams Ranch, TX	11

NWR, National Wildlife Refuge.

AF083371). In addition, nucleotide sequence from a single representative of the Charolais breed (*B. taurus*), two individuals from each of six additional *B. taurus* breeds (Angus, Hereford, Jersey, Friesian, N'Dama, and Simmental), and two individuals from each of five additional *B. indicus* breeds (Butana, Kenana, Hariana, Sahiwal, and Tharparker) was kindly provided by D. Bradley (personal communication). The region examined corresponds to that between nucleotides 15,854 and 188 in the domestic cow genome (Anderson *et al.* 1982).

Total genomic DNA was isolated from white blood cells using the SUPER QUICK-GENE DNA isolation kit (Analytical Genetic Testing Center Inc.) or by proteinase K treatment followed by organic extraction (Sambrook *et al.* 1989). Alternatively, DNA was extracted from hair follicles digested for four hours at 55° in 200µl lysis buffer (500mM KCl, 100mM Tris-HCl pH 8.0, 0.1µg/ml Geletin, 0.45% Triton X-100, 0.45% Tween 20, 0.5 mg/ml proteinase K). After digestion, samples were centrifuged at 5,000g for two minutes, and the clear aqueous layer was incubated at room temperature for 10 minutes with 10mg/ml RNase A (R. Schnabel, personal communication). Organic extractions were then performed as described in Sambrook *et al.* (1989).

The entire control region was amplified with oligonucleotide primers located in the flanking threonine tRNA (5′-AGAGAAGGAGAACAACTAACCTCC-3′, 15695) and 12s rRNA (5′-AACAGGAAGGCTGGGACC-3′, 457) genes using the polymerase chain reaction (PCR) (Saiki *et al.* 1988). The numbers after the primer sequences indicate the position of the 5′ nucleotide in the domestic cow mitochondrial genome (Anderson *et al.* 1982). Amplification was performed with a modified version of the touchdown PCR method of Don *et al.* (1991), and consisted of 45 sec at 94° (denaturation), 45 sec at 60° (annealing), and 45 sec at 74° (extension) in the presence of 2.5mM $MgCl_2$ and 2.5 U of Taq polymerase (Promega). The annealing temperature was lowered 1° every two cycles until it reached 55°, which was used for five cycles. After this, the annealing temperature was dropped to 52° for the remaining 20 cycles of a 35 cycle complete run. Amplification products were resolved on 1.5% agarose gels and purified with QIAquick gel extraction columns (Qiagen).

Nucleotide sequences were determined using a dye terminator cycle sequencing kit with Amplitaq DNA polymerase FS and an ABI PRISM 377 automated DNA sequencer (Applied-Biosystems, Perkin Elmer). Sequence data was verified by completely

sequencing both strands of the PCR products. Sequences were aligned using Clustal V (Higgins and Sharp, 1989), with homologous alignment verified by manual inspection.

Estimates of nucleotide divergence were calculated with the program MEGA (Kumar *et al.* 1993), using the Tamura and Nei (1993) correction, which was specifically developed for mitochondrial control region sequences. Because the rate of nucleotide substitution is known to vary extensively from site to site in mammalian control region sequences (Kumar *et al.* 1993), a correction for among-site rate heterogeneity was incorporated using a continuous gamma distribution. The maximum likelihood estimate of the shape parameter (alpha) was determined using the method of Yang (1994) as implemented in PAUP* version 4.0d63 (PAUP*) (Swofford 1998). In order to simplify this analysis, only one individual from each domestic cattle breed was included in these comparisons. In addition, individuals from the two African *B. indicus* breeds (Butana and Kenana) were excluded from this analysis because of their problematic taxonomic placement (Loftus *et al.* 1994).

MacVector 5.0 (International Biotechnologies Inc.) was used to examine nucleotide sequences for primer sites that were conserved across all 14 breeds of domestic cattle and that were highly degenerate in wild cattle and bison species. The resulting oligonucleotide primers (5′-AGCTAACATAACACGCCCATAC-3′, 15907 and 5′-CCTGAAGAAAGAACCAGATGC-3′, 16264) were used in multiplex PCR reactions with highly conserved primers located in the 16s rRNA gene (5′-CCCGCCTGTTTATCA AAAACAT-3′, 2284 and 5′-CCCTCCGGTTTGAACTCAGATC-3′, 2878) (Derr *et al.*, 1992). Samples for which the control region product amplified were considered to have domestic cattle mtDNA haplotypes, while the presence of a 590 bp 16s rRNA product served as an internal positive control for each individual reaction (Figure 1). Amplifications consisted of 35 cycles of 30 sec at 94° (denaturation), 30 sec at 55° (annealing), and 1 min at 74° (elongation), in the presence of 2.0 mM $MgCl_2$ and 2.5 U of Taq polymerase (Promega). However, efficient amplification of some less well preserved samples required slightly less stringent reaction conditions (54° annealing temperature and 2.5 mM $MgCl_2$).

In order to verify the results of the genetic test, mtDNA control region sequences were determined for all individuals identified as domestic cattle hybrids. Maximum parsimony analysis of all cattle and bison haplotypes identified, was conducted with the tree-bisection and reconnection (TBR) method of branch swapping and the heuristic search algorithm of PAUP* (Swofford 1998). Maximum likelihood analysis was also conducted

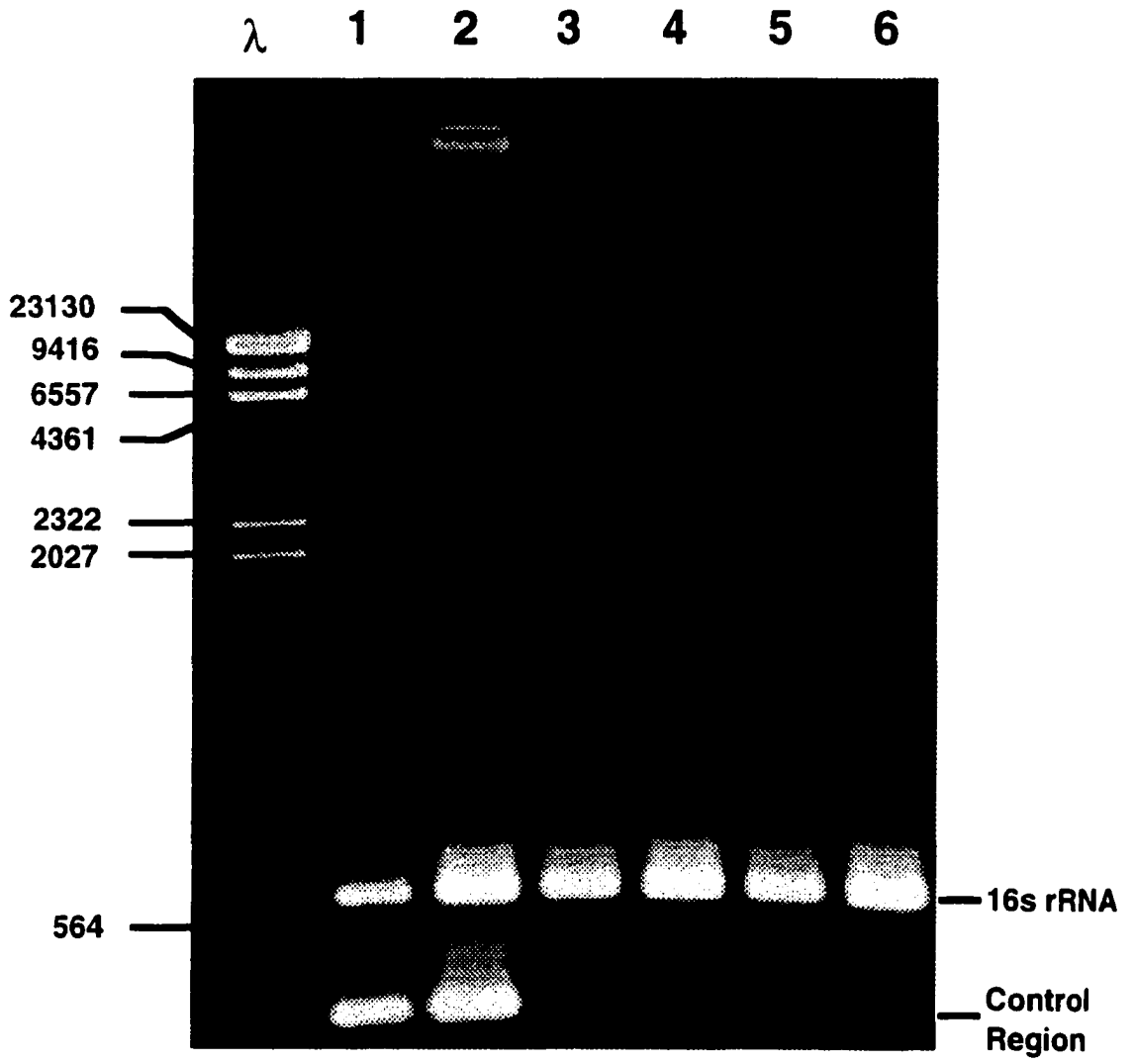

Figure 1. -Results of duplex PCR amplification of DNA from (1) *Bos taurus*, (2) *Bos indicus*, (3) *Bison bison*, (4) *Bison bonasus*, (5) *Bos grunniens*, and (6) *Bos gaurus* using mtDNA control region and 16s rRNA primers. Samples that produced the 357 bp mtDNA control region product possess domestic cattle mtDNA haplotypes.

with the (TBR) method of branch swapping and the heuristic search algorithm of PAUP* (Swofford 1998). However, due to the computational complexity of maximum likelihood analysis, a reduced data set of representative haplotypes sampled from the various clades of the parsimony tree was used. The model of Tamura and Nei (1993) was chosen for the maximum likelihood analysis, and a discrete approximation to the gamma distribution (TN+dG_n model) with the number of rate categories determined by the method of Yang (1994), was applied to account for among site rate heterogeneity. The explanatory power of this model was assessed with the generalized likelihood ratio test of Goldman (1993) in order to determine if it provided an adequate representation of the pattern of molecular evolution for the sampled cattle and bison sequences.

The presence of haplotypes from wild cattle and bison species within domestic cattle clades of optimal parsimony or likelihood trees was considered as evidence of interspecific hybridization. Each of the hybridization events indicated by the phylogenetic trees (paraphyly) was treated as a separate hypothesis and was tested against a corresponding null hypothesis of monophyly, using the parametric bootstrap method as described by Hillis and Huelsenbeck (1994) and by Huelsenbeck *et al.* (1996). The optimal tree and model parameter estimates under each hypothesis of monophyly were obtained using maximum likelihood by constraining the presumably hybrid and native haplotypes identified in a given species to form a monophyletic group. The maximum likelihood estimates of the constraint tree topology (model tree), branch lengths, and substitution parameters were then used to simulate 100 data sets with the program Seq-Gen 1.04 (Rambaut and Grassly 1997). By analyzing each simulated data set using maximum likelihood and computing the difference in likelihood scores between the optimal tree for that data set and the tree used to simulate that data set (model tree), the expected distribution of difference scores was obtained. This distribution was used to test the possibility that the non-monophyletic clades (consistent with hybridization) obtained from phylogenetic analyses resulted from systematic error when monophyly was true.

Results

Observed levels of intraspecific variation were insufficient to negatively impact the discriminatory power of the genetic test. Mean intraspecific divergence was $0.89 \pm 0.54\%$ and $0.71 \pm 0.45\%$ respectively among the eight European and four Indian cattle breeds examined. The average genetic distance between North American bison haplotypes was $0.69 \pm 0.27\%$, while no variation was found among the three European bison or two yak

sequences examined. Alternatively, large estimates of interspecific divergence between domestic cattle and wild cattle or bison species were obtained, and ranged from 19.00 ± 1.18% to 29.53 ± 1.37% (Table 2). Interestingly, the divergence between North American bison and yak (12.28 ± 0.41%) was lower than expected. relative to that between the two bison species (17.73 ± 0.57%), which suggested that an ancient hybridization event or phylogenetic lineage sorting may have taken place (Janecek *et al.* 1996). However, these estimates of genetic divergence, as well as those shown in Table 2, indicated that the haplotypes used to design domestic cattle specific oligonucleotides were not present in a given species due to recent interspecific hybridization.

The development of domestic cattle specific oligonucleotide primers provided a rapid method of differentiating between domestic cattle and wild cattle or bison mitochondriai haplotypes. As a result, one of the four European bison from Russia, and one of the three yak (Kansas City Zoo) were identified as having domestic cattle mtDNA. In addition, domestic cattle mtDNA haplotypes were found in seven of the 16 (43.8%) North American bison populations and 36 of the 610 (5.9%) bison tested. All seven of the North American bison populations where domestic cattle mtDNA haplotypes were identified are of the plains bison subspecies.

Animals identified with the genetic test as having domestic cattle mtDNA were further examined by determining the nucleotide sequence of the 677 bp control region segment as described above. Based on these sequences, four distinct haplotypes were identified among the 36 North American bison found to have domestic cattle mtDNA (Table 3). One of these haplotypes was unique to Custer State Park in South Dakota, where seven of the 34 (20.6%) bison tested had domestic cattle mtDNA. Another domestic cattle haplotype was found only in bison from the Williams ranch in west Texas, where all 11 of the animals tested had domestic cattle mtDNA. This haplotype was identical to a haplotype found in both Friesian and Hereford breeds of domestic cattle. A third haplotype was shared among bison from Custer State Park, Caprock Canyon State Park, and the Williams Ranch populations. This haplotype was identical to a haplotype found in Friesian and Simmental breeds of domestic cattle. The fourth haplotype was present in all bison with domestic cattle mtDNA from the Maxwell (7 of 39) and Finney (1 of 26) State Game Refuges in Kansas as well as those from the National Bison Range (3 of 113) in Montana and Antelope Island State Park (1 of 95) in Utah.

The four mtDNA haplotypes from North American bison identified as originating from domestic cattle had an average genetic distance of 19.36 ± 1.04% from the nine

TABLE 2

Percent nucleotide divergence, corrected using the Tamura-Nei model (above diagonal) with between site rate heterogeneity (below diagonal, alpha = 0.15)

	Bos taurus	*Bos indicus*	*Bos grunniens*	*Bos gaurus*	*Bison bonasus*	*Bison bison*
Bos taurus	-	5.42 ± 0.28	12.10 ± 0.26	11.53 ± 0.25	9.77 ± 0.34	9.91 ± 0.24
Bos indicus	8.18 ± 0.63	-	11.17 ± 0.14	12.25 ± 0.18	10.25 ± 0.35	10.47 ± 0.43
Bos grunniens	29.53 ± 1.37	24.23 ± 0.56	-	10.25 ± 0.00	8.82 ± 0.00	7.36 ± 0.13
Bos gaurus	25.81 ± 1.04	28.20 ± 1.00	21.60 ± 0.00	-	12.83 ± 0.00	10.47 ± 0.28
Bison bonasus	19.00 ± 1.18	21.06 ± 1.20	16.59 ± 0.00	28.70 ± 0.00	-	9.44 ± 0.17
Bison bison	19.72 ± 0.85	24.73 ± 1.22	12.28 ± 0.41	21.16 ± 1.14	17.73 ± 0.57	-

TABLE 3

Haplotypic frequency data for North American bison

	1	2	3	4	5	6	7	8	9*	10*	11*	12*	13
Elk Island National Park (W)				3				1					
Mackenzie Bison Sanctuary (W)			1	2				2					
Wood Buffalo National Park (W)			2	1	1								
Antelope Island State Park					4				1				
Custer State Park						1		1			4	3	
Elk Island National Park	1					2	1	1					
Finney Game Refuge									1				
Fort Niobrara NWR						5							
Henry Mountains						5							
Maxwell Game Refuge									7				
National Bison Range						1	2	2	3				
Wind Cave National Park						5							
Witchita Mountains NWR		1				3							
Yellowstone National Park						3		2					
Caprock Canyon State Park											6		6
Williams Ranch										2	9		
Total Number of Individuals	1	1	3	6	5	25	3	9	12	2	19	3	6

Haplotype 13 was identified after the publication of haplotypes 1-12 in Ward *et al.* (1999). *, haplotypes identified as having a domestic cattle origin; (W), wood bison populations; NWR, National Wildlife Refuge.

native North American bison haplotypes identified, and a difference of only 0.77 ± 1.10% from the eight European cattle breeds examined. Similarly, the European bison identified by the genetic test as having domestic cattle mtDNA differed by 16.91 ± 0.00% from the other European bison that were sequenced, but only by 0.87 ± 0.44% from the eight European cattle breeds examined. Finally, the single yak identified as having domestic cattle mtDNA differed by 26.85 ± 0.00% from the other yaks examined, but only by 0.69 ± 0.45% from the four Indian cattle breeds.

The results of phylogenetic analysis corroborated the conclusion that mtDNA haplotypes from the North American bison, European bison, and yak, identified with the genetic test as having domestic cattle mtDNA, were of domestic cattle origin. In the maximum parsimony result, the domestic cattle haplotypes identified in North American bison and European bison were located within a largely unresolved clade of European and African cattle breeds, while the domestic cattle haplotype identified in yak was located within a clade of Indian cattle breeds (Figure 2). Maximum likelihood analysis was conducted using the TN+dG_4 model of molecular evolution, which was accepted as having adequate explanatory power because it did not provide a significantly worse fit to the data than the theoretically perfect fit of the multinomial (Figure 3). The maximum likelihood analysis resulted in a single tree (Figure 4) which was completely congruent with the results from maximum parsimony analysis, and thus with the hypotheses of hybridization.

Constraining the maximum likelihood topology so that European bison, North American bison, or yak haplotypes were monophyletic resulted in likelihood scores that were greater (less optimal) than that of the global maximum likelihood topology (Figure 3) by 17, 28, and 34 units respectively. Using the parametric bootstrap method, it was determined that the probability of obtaining likelihood score differences as great as those observed, when the null hypothesis of monophyly was true, was $P < 0.05$ for all three hypotheses of monophyly (Figure 5). Therefore, the hypotheses of monophyly were rejected and the hypotheses that domestic cattle introgression has occurred in North American bison, European bison, and yak were accepted as significantly better explanations of the observed data.

A good deal is known about the histories of the North American bison populations in which domestic cattle mtDNA haplotypes were identified. For instance, Custer State Park (CSP) was derived from the Dupree herd (Dary 1989), where hybridization with domestic cattle is known to have occurred (Coder 1975). The CSP population is unrelated to any of the other populations where domestic cattle mtDNA haplotypes were identified,

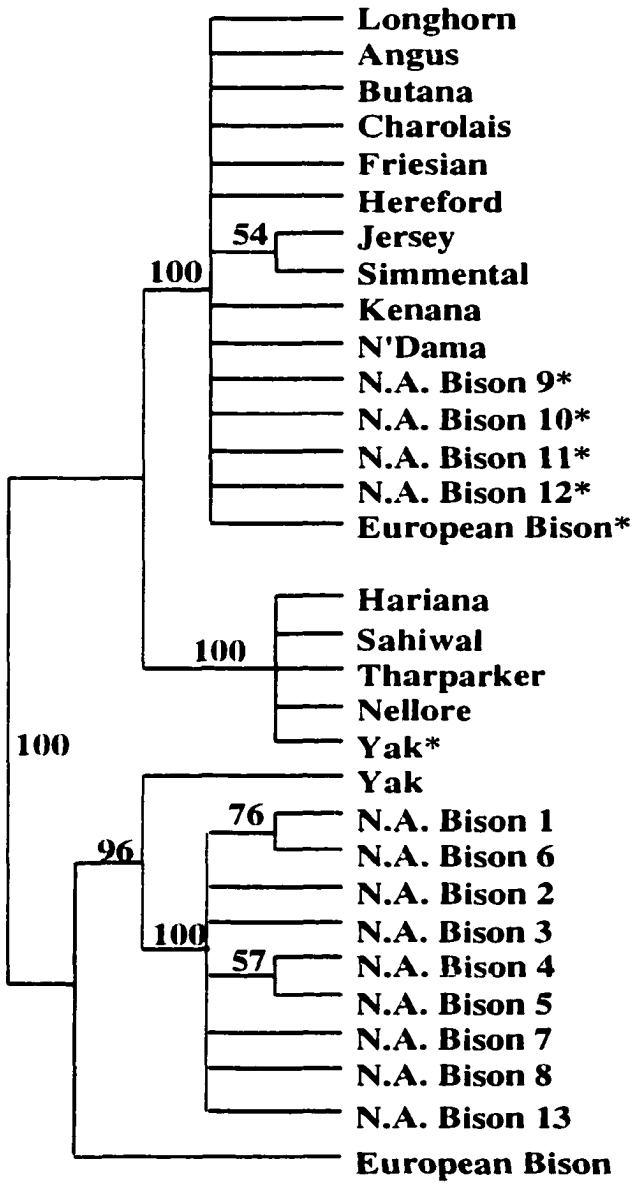

Figure 2. -Majority rule bootstrap consensus tree from maximum parsimony. The tree score = 189 steps, the consistency index (excluding uninformative characters) = 0.62, and the retention index = 0.92. The frequency (%) with which a given branch was recovered in 2000 bootstrap replications is shown above all branches recovered in more than 50% of replicates. Haplotypes from wild cattle and bison that were determined by the genetic test to have a domestic cattle origin are marked with an asterisk (*).

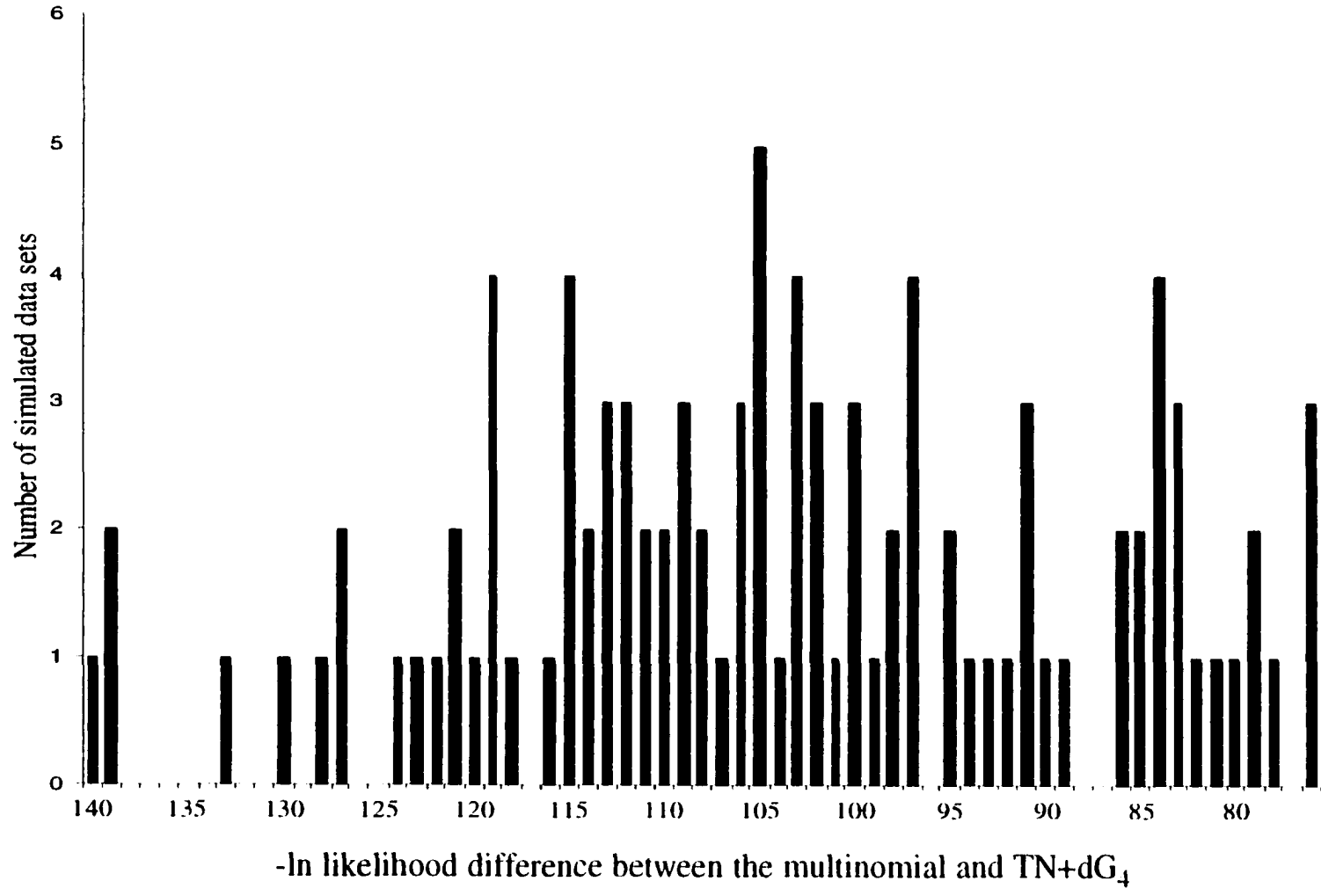

Figure 3. -Distribution of likelihood score differences for the generalized likelihood ratio test of model fit. The critical value ($P < 0.05$) = 130 likelihood units. The observed difference = 106 likelihood units.

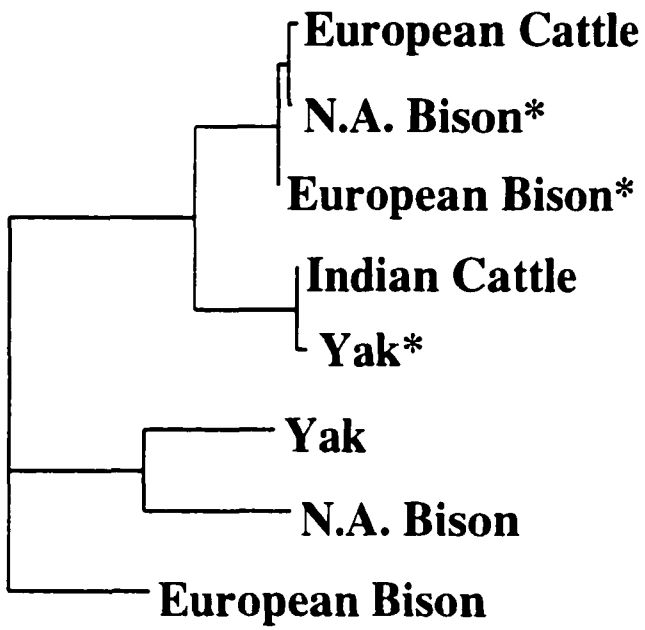

Figure 4. -Maximum likelihood topology constructed using the TN+dG$_4$ model of molecular evolution. The -ln likelihood for this tree = 1550.94951. Haplotypes from wild cattle and bison that were determined by the genetic test to have a domestic cattle origin are marked with an asterisk (*).

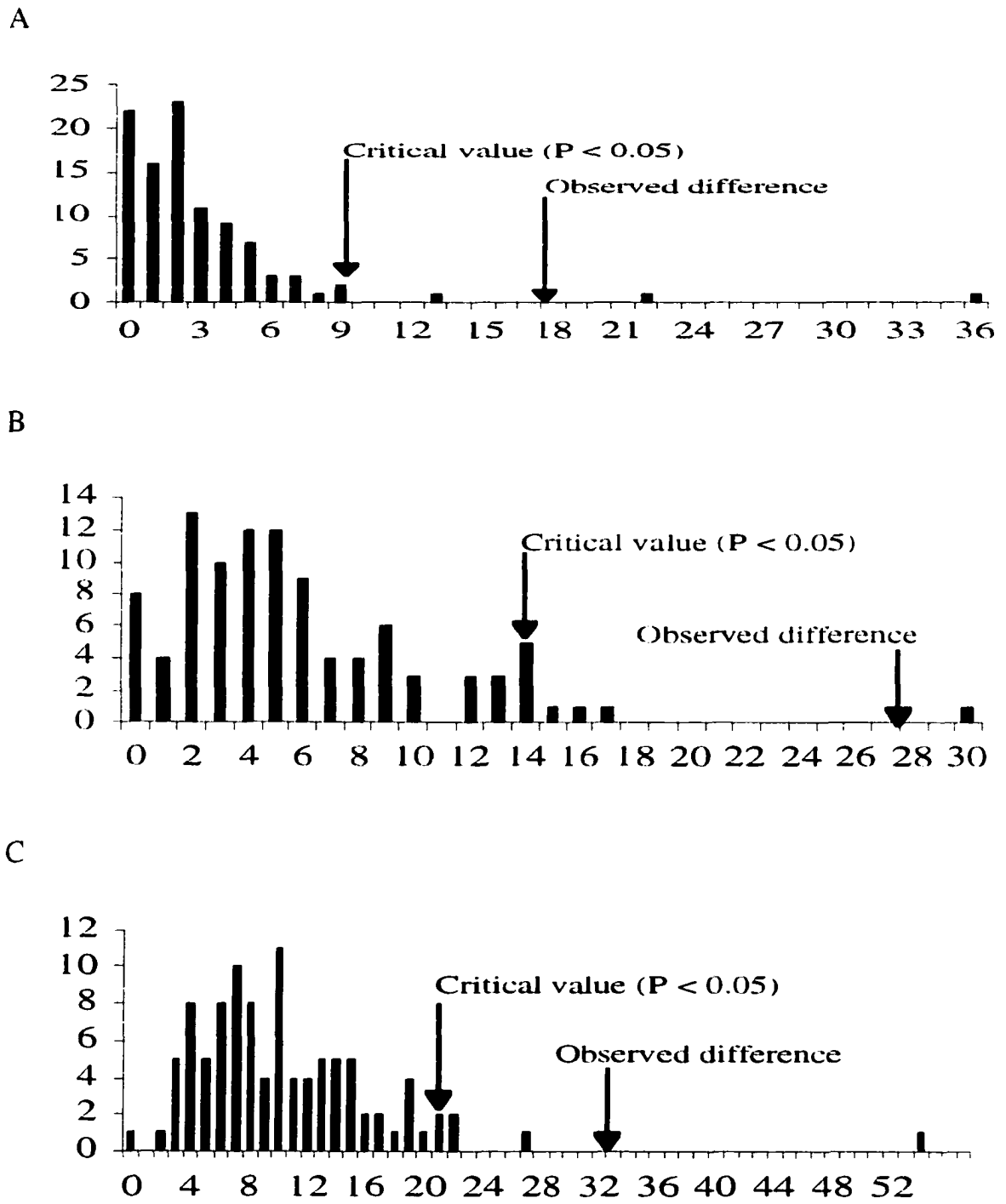

Figure 5. -Distribution of -ln likelihood score differences for optimal versus model trees, with the null hypothesis being (A) European bison monophyly, (B) North American bison monophyly, and (C) yak monophyly. The difference in -ln likelihood between optimal and model trees is shown along the x axis, with the number of simulations shown along the y axis.

indicating that the CSP population has a direct history of hybridization, independent of these other populations. Similarly, North American bison from the Caprock Canyon State Park (JA) population were recently used as the sole stock for the formation of the Williams ranch (CW) population, and are the direct descendants of the Goodnight herd (D. Swepston, personal communication). These two populations are the last known populations of southern plains bison and are unrelated to any of the other populations where domestic cattle mtDNA haplotypes have been identified. Hybridization was known to have occurred in the Goodnight population (Coder 1975), indicating that the JA and CW populations have a direct history of hybridization, and represent a second independent hybridization event.

Park records (D. Wiseman and K. Sherman, personal communication) and the haplotype data presented in Table 3 were used to determine that the North American bison with domestic cattle mtDNA haplotypes present in the National Bison Range (NBR) and Antelope Island State Park (AISP) populations are the result of recent transfers from the Maxwell State Game Refuge (MSGR) population. The same domestic cattle haplotype that was found in North American bison from the NBR, AISP, and MSGR populations is also seen in the Finney State Game Refuge (FSGR) population (Table 3). Precise records on this population are not available, but both the FSGR and MSGR populations are in Kansas, and they have a closely connected history (T. Norman, personal communication). In addition, these populations have no known connection with the CSP, JA, or CW populations. Therefore, while the precise population of origin for this haplotype can not be identified, it appears that the FSGR and MSGR populations in Kansas, or an unidentified common source population, represent a third independent center of mtDNA introgression. Therefore, the haplotype data presented in Table 3, along with historical and recent park records, indicate that there are at least three independent mitochondrial introgression events represented in current populations of North American bison.

Discussion

The results of screening wild cattle and bison species with the PCR-based genetic test indicated that hybridization between domestic cattle and North American bison, European bison, and yak had occurred. This conclusion was supported by phylogenetic analyses in which parametric bootstrap results demonstrated that a topology consistent with hypotheses of interspecific hybridization had significantly ($P < 0.05$) greater support than did topologies in which North American bison, European bison, or yak haplotypes

were monophyletic. While the results of these phylogenetic analyses were consistent with interspecific hybridization, non-phylogenetic lineage sorting can also result in conflicts between gene trees and species trees. However, the fast rate of reciprocal monophyly for mtDNA markers (Moore 1995) and the extremely small genetic distances between presumably hybrid haplotypes and those of domestic cattle make the hypothesis of lineage sorting implausible. In addition, the observation that domestic cattle introgression in the two bison species involved breeds from the European and African domestic cattle lineage, while Indian cattle breeds were involved in hybridization with yak, is consistent with historical accounts of hybridization events (Jones 1907; Boyd 1914; Goodnight 1914; National Research Council 1983), and with the geographic distributions of the two bison species, yak, and the two domestic cattle species (Macdonald 1984; Loftus *et al.* 1994). Therefore, the results of this study indicate that independent hybridization events have occurred between domestic cattle and North American bison, European bison, and yak.

For threatened or publicly supported species, the identification of populations with hybrid ancestry leads to the question of how to deal with these populations in the future management of the species. The simplest approach to dealing with interspecific hybridization is to simply ignore it, thus accepting some level of genetic introgression as inevitable. This may be the only real option for endemic or highly threatened species which consist of only a few remaining individuals or a single small population. However, this would be an unsatisfactory approach for species with less extreme circumstances, and could result in the introduction of hybrid individuals into genetically pure populations, as appears to have happened for the National Bison Range and Antelope Island State Park bison herds.

For many species, the most practical approach to dealing with introgression is to use genetic markers, such as those developed here, to identify populations with hybrid ancestry and eliminate them from conservation efforts such as captive breeding and reintroduction programs. This would prevent the problem of interspecific hybridization from being exacerbated by the introduction of hybrid individuals into genetically pure populations. However, this strategy is only possible when multiple populations exist, and when remaining populations have large enough effective population sizes to prevent the erosion of genetic diversity. Therefore, accurate ecological and population genetic information will be required in order to determine if this approach is appropriate for a given species.

Full assessment of the impact of domestic cattle introgression on North American bison, European bison, and yak populations will require information from multiple independent nuclear loci, in addition to mtDNA data, so that all populations with hybrid individuals can be identified, and the frequency of domestic cattle genes within these populations can be determined. In addition, because the number of European bison and yak examined was limited by the difficulty in obtaining samples from these relatively rare and exotic animals, additional genetic and demographic information will be required to determine the extent of domestic cattle introgression in these species and the appropriate measures for dealing with this issue in future conservation efforts. In the case of North American bison however, there are a number of fairly large populations for which there is no evidence of domestic cattle introgression. Therefore, it seems prudent to maintain these populations in isolation from those with hybrid ancestry until a more complete picture of the impact of this hybridization can be developed. Even if the expected levels of introgression are low, it seems unwise to allow animals with hybrid ancestry to be transferred into presumably pure populations if other options are available.

As indicated by the wild cattle and bison examples, human activities may be increasing the opportunities for interspecific hybridization in many species. Attempts to deal with interspecific hybridization can be confounded by other problems faced by threatened species, such as the loss of genetic variability and localized population extinction. Therefore, because the consideration of genetic purity in conservation plans will generally involve trade-offs with other aspects of species conservation, the application of information gained about hybrid animals is necessarily dependent on the individual circumstances of the species examined. However, by examining individual cases of introgressive hybridization, a better understanding of its role in evolution and its impact on small populations can be obtained. The identification of individuals or populations with a hybrid ancestry is a necessary first step in developing such an understanding. Therefore, generally applicable approaches for detecting hybrid individuals, and rigorous statistical methods of documenting introgression, such as those presented here, are required for making appropriate conservation decisions, and ultimately for understanding the evolutionary significance of hybridization in general.

DISEQUILIBRIUM MAPPING A HYBRID GENOME: THE IDENTIFICATION OF DOMESTIC CATTLE CHROMOSOMAL SEGMENTS IN NORTH AMERICAN BISON

Synopsis

The benefits of using linked molecular markers in studies of introgression have been recognized by many authors. However, few studies have made use of the superior genealogical information derived from the addition of linked molecular markers in actual investigations of introgression. In this study, domestic cattle introgression in North American bison populations is examined using 22 diagnostic microsatellite markers, representing 12 autosomes and both the X and Y chromosomes. These diagnostic microsatellite loci were identified from a panel of 100 microsatellite markers obtained from domestic cattle genome maps. By combining genotypes from closely linked microsatellite markers with those from the 22 diagnostic markers, domestic cattle nuclear introgression was identified in five of the 14 (35.7%) North American bison populations examined. Domestic cattle introgression was identified in seven different genomic regions, including a region containing a locus known to effect growth and conformation characters in domestic cattle.

Introduction

Questions regarding the evolutionary significance of introgressive hybridization have gained renewed interest due to the increasing opportunities for interspecific hybridization created by human induced habitat alteration and population fragmentation (Avise 1994; Rhymer and Simberloff 1996; Arnold 1997). In order to address these questions, cases of introgressive hybridization must first be documented. However, the identification of cases of introgressive hybridization can be hampered by several practical problems. For instance, the potential for hybridization generally decreases with increasing levels of divergence between two taxa, while the probability of detecting introgression is greatest at higher levels of divergence, where there are likely to be a greater number of diagnostic characters distinguishing the taxa involved (Riesberg and Wendel 1993). The difficulty of identifying introgression events is evidenced by a recent review, in which Riesberg and Wendel (1993) determined that there have been only 65 well documented cases of introgression in plants. This is a surprisingly low number given the fact that

hybridization is often thought to be a general feature of plant evolution (Lotsy 1931; Heiser 1973; Arnold 1997).

The advent of molecular technologies has greatly facilitated the identification of introgressive hybridization events. This is largely due to the fact that diagnostic differences are more likely to be found among neutral molecular markers than in phenotypes displayed by two closely related taxa. Traditionally, molecular investigations of hybridization have relied upon cytoplasmic or allozyme markers (Avise and Saunders 1984; Harrison *et al.* 1987; Gyllenstein and Wilson 1987; Klier *et al.* 1991; Rhymer *et al.* 1994; Seeb 1998). However, a detailed characterization of the outcome of introgressive hybridization events requires a large number of diagnostic molecular markers distributed throughout the genome. Microsatellite markers offer a significant advantage over other types of molecular markers because of their abundance and seemingly ubiquitous distribution throughout eukaryotic genomes (Hamada *et al.* 1982; Tautz and Renz 1984; Gyapay *et al.* 1994). In addition, the rapid mutation rate displayed by microsatellite loci (Dallas 1992; Edwards *et al.* 1992) decreases the potential that alleles shared in common between two taxa are the result of descent from a common ancestor (symplesiomorphy). The rapid rate of mutation also increases the probability that diagnostic differences between taxa will develop in the course of divergent evolution.

Several features of the molecular evolutionary dynamics of microsatellite loci complicate their application to investigations of introgressive hybridization. For instance, the pattern of molecular evolution at microsatellite loci conforms to a stepwise mutation model, in which mutations result in the loss or gain of single repeat units (Shriver *et al.* 1993; Valdes *et al.* 1993). A stepwise pattern of molecular evolution in combination with a rapid rate of mutation greatly increases the probability of allele size homoplasy, which results when alleles with different evolutionary histories display the same electrophoretic mobility (size) because of parallel or reverse mutations. If shared alleles are assumed to be the result of recent introgression due to the low probability of symplesiomorphy, then levels of introgression may be overestimated because of allele size homoplasy. This problem is potentially exacerbated by the observation that there may be allele size limits at microsatellite loci (Garza *et al.* 1995), which have been shown to increase the probability of allele size homoplasy, even in intraspecific comparisons (Nauta and Weissing 1996).

The availability of information on the linkage relationships between different microsatellite loci makes it possible to overcome inherent difficulties in their use as molecular markers for identifying introgression. Several authors have previously noted

that the use of tightly linked molecular markers in studies of introgression makes it possible to differentiate between symplesiomorphy, allele size homoplasy, and introgression (Avise and Saunders 1984; Doebley 1989; Riesberg and Wendel 1993; Riesberg *et al.* 1995). More recently, Estoup *et al.* (1999) demonstrated theoretically, that the use of juxtaposed microsatellite systems (pairs of microsatellites separated by less than 200 bp) can overcome problems associated with allele size homoplasy and symplesiomorphy, because of the superior genealogical information contained in two tightly linked microsatellite sites as opposed to a single microsatellite marker. The advantage of using linked markers is derived from the fact that the presence of a presumably alien allele (shared allele present as a result of introgression) at a given locus is complemented by the presence of another presumably alien allele at a second tightly linked locus in the same individual. Thus, the chances that symplesiomorphy or allele size homoplasy could be mistaken for introgression are greatly reduced (Riesberg and Wendel 1993).

While the use of juxtaposed microsatellite systems (JMS) as defined by Estoup *et al.* (1999) provides an excellent method for detecting introgression using microsatellite markers, this approach need not be limited to markers with such extreme linkage relationships (separated by less than 200 bp). When a hybridization event occurs, syntenic markers from each of the parent species are initially in complete disequilibrium. This disequilibrium will decay as a function of time due to recombination. However, disequilibrium between markers separated by as much as five centimorgans (cM) can be expected to persist for as much as 20 generations after the hybridization event (Barton and Gale 1993). Therefore, a generalized form of the JMS, using microsatellite loci spanning many centimorgans, would provide the superior genealogical information derived from the use of JMS, and would greatly expand the percentage of the genome that could be examined. Using this approach, the differential introgression of whole chromosomes or chromosomal segments can be identified.

Hybridization between North American bison and domestic cattle at the end of the nineteenth century has been well documented (Jones 1907; Boyd 1914; Goodnight 1914; Coder 1975; Dary 1989). The large amount of information available about the hybridization events themselves, along with the availability of a well defined linkage map for domestic cattle, provides a unique framework for examining the utility of a disequilibrium mapping approach in the identification and characterization of introgression events. As described in the previous chapter, domestic cattle mtDNA haplotypes have

been identified in a number of North American bison populations. Wilson and Strobeck (1999) reported that they found no evidence of nuclear introgression in one of the populations where domestic cattle mtDNA introgression had been documented (Polziehn *et al.* 1995, Ward *et al.* 1999). However, this inference was based on an examination of 11 microsatellite markers with alleles in North American bison that were almost entirely shared by domestic cattle. Obviously, a serious evaluation of the outcome of historically documented hybridization between North American bison and domestic cattle requires an examination of introgression at multiple nuclear loci for which the two species share no native alleles in common (diagnostic loci).

The application of a disequilibrium mapping approach allows introgression to be differentiated from symplesiomorphy and allele size homoplasy at microsatellite loci, and therefore, makes available the large number of diagnostic loci needed to characterize the outcome of hybridization events. This chapter describes the identification of a panel of diagnostic microsatellite markers and demonstrates the utility of using linked microsatellite markers for identifying populations which have a history of introgressive hybridization. Using this approach, the frequency and genomic distribution of domestic cattle introgression in North American bison populations is determined.

Materials and Methods

A total of 100 microsatellite markers (Table 4) were evaluated for their utility in identifying domestic cattle introgression in North American bison. It should be noted that SPS113 and BM4513 were selected for evaluation because previous studies indicated that they might be useful for examining introgressive hybridization between domestic cattle and North American bison (Mommens *et al.* 1998; Wilson and Strobeck 1999). In order to facilitate the evaluation of this large number of microsatellite markers, forward primers for each locus were synthesized with one of three different fluorescent labels (Applied-Biosystems, Perkin Elmer), and the amplification of markers was performed in multiplex polymerase chain reactions (PCR) whenever possible. Amplification conditions for all of the markers examined are given in Appendix A. Genotyping was performed on an ABI377 Automated DNA Sequencer or an ABI310 Gene Analyzer (Applied-Biosystems, Perkin Elmer) using GENESCAN 3.1 software (Applied-Biosystems, Perkin Elmer). Genotypes were assigned using GENOTYPER 2.0 software (Applied-Biosystems, Perkin Elmer) and either a GS500 TAMRA (Applied-Biosystems, Perkin Elmer) or a MAPMARKER LOW TAMRA (Bioventures Inc.) internal size standard.

TABLE 4

Microsatellite loci that were screened in order to identify diagnostic markers for examining introgression

Locus	Chromosome	Position (cM)	DC Range	WBNP Range	YNP Range	Amplification Protocol
IFNAR15-2 [15]	1	0.7	159-161	167-167	167-167	17
BMS574 [1]	1	14.0	129-145	135-141	131-141	18
PIT1 7B7 [15]	1	35.0	128-143	145-159	143-157	28
TGLA57 [2]	1	46.2	82-102	88-102	88-100	7
BMS527 [1]	1	55.9	167-189	163-177	163-177	3
BM7145 [2]	1	69.2	115-117	107-109	107-109	21
BMS4023 [2]	1	75.0	105-111	105-107	105-107	25
BMS4031 [2]	1	77.7	291-299	287-297	287-297	14
BMS4040 [2]	1	98.8	84-98	74	74	16
BM1824 [3]	1	108.6	180-192	178-198	180-198	12
CSSM42 [4]	2	34.4	173-217	167-171	167-171	22
BMS803 [1]	2	41.0	131-147	129-131	129-131	21
BM4440 [3]	2	55.0	123-149	121-139	123-129	4
BMS2 [1]	2	61.6	141-145	141	141	21
NRAMP1 [2]	2	88.4	155-163	Null	Null	25
BM2113 [3]	2	106.2	127-143	127-149	129-149	3
HUJ246 [6]	3	67.9	242-258	256-262	258-264	11
BMC4214 [2]	3	123.0	172-214	174-191	174-187	19
BMS827 [1]	4	24.7	91-105	89-91	89-91	13
BMS1172 [1]	4	27.3	86-96	88-102	88-104	2
BMS2809 [2]	4	72.0	144-171	140-142	142-142	11
BMS1074 [1]	4	74.9	140-157	149-157	151-157	24
TCRB [2]	4	97.2	123-127	140-146	144-146	25
BMS1095 [1]	5	0.0	95-119	95-97	95-97	19
BMS610 [1]	5	12.8	104-128	104-109	104-109	18
BL23 [3]	5	28.6	242-256	234-236	234-234	23
RM500 [5]	5	55.6	122-132	120	120	8
CSSM22 [4]	5	71.1	218-226	205-232	218-238	17
BM2830 [3]	5	120.2	151-174	148-168	148-166	10

TABLE 4 CONTINUED

Locus	Chromosome	Position (cM)	DC Range	WBNP Range	YNP Range	Amplification Protocol
BMS360 [1]	6	66.5	77-107	79-91	81-91	12
ILSTS035 [9]	6	81.0	211-239	228-234	228-230	18
CSN3 [11]	6	82.6	215-225	215-217	215-221	21
BM4311 [3]	6	89.7	92-106	92-104	90-104	10
BMS1116 [1]	7	30.5	141-143	141-143	139-143	6
IL4 [7]	7	30.5	85-97	83-103	83-105	14
BMS2840 [2]	7	64.3	218-266	246-276	246-270	10
BMS2258 [2]	7	75.0	132-152	127-150	127-150	12
BMS1979 [1]	7	124.4	94-110	92-96	90-96	12
RM372 [2]	8	19.1	116-134	114-134	114-136	2
BM757 [3]	9	0.6	187-219	188-201	188-201	17
BMS555 [1]	9	39.1	158-176	156-158	156-158	6
BMC701 [3]	9	56.7	277-305	275-285	265-283	15
BMS2295 [1]	9	91.5	108-131	112-120	112-120	21
BMS1967 [1]	9	102.5	82-105	76-95	76-95	14
BMS528 [1]	10	19.0	144-170	140-152	140-152	18
BMS2349 [2]	10	22.1	69-114	86-90	86-94	16
SPS113 [14]	10	29.2	134-153	129-131	129-131	19
BMS419 [1]	10	51.1	142-160	142-154	142-156	15
CSSM46 [4]	10	92.9	163-185	154-158	143-154	7
BMS1716 [2]	11	47.7	187-217	189-195	189-195	11
BMS2047 [1]	11	73.8	147-221	140-143	141-142	14
BM9146 [1]	11	74.1	162-168	160-168	160-168	26
BMS460 [1]	11	102.6	120-133	120-125	120-125	5
BMS410 [1]	12	0.0	83-107	81-95	83-97	2
BMS585 [1]	12	79.7	126-134	126-140	126-128	7
BM4028 [3]	12	79.7	101-126	106-117	106-117	18
BM720 [3]	13	38.6	210-240	224-230	214-234	4
ILSTS008 [10]	14	35.2	94-104	94-104	102-104	19
BMS947 [1]	14	52.8	107-131	101-112	101-109	9
BM4513 [3]	14	62.5	135-162	128	128-130	9

TABLE 4 CONTINUED

Locus	Chromosome	Position (cM)	DC Range	WBNP Range	YNP Range	Amplification Protocol
BM2934 [3]	14	66.4	76-98	80-82	80-82	13
BMS2055 [1]	14	84.1	143-164	143	143	19
BMS2533 [2]	15	5.2	128-161	132-138	132-138	10
BMS1004 [1]	15	7.2	143-165	143-147	143-145	20
BMS820 [1]	15	81.6	110-132	110-114	110-114	14
BM121 [3]	16	24.4	118-160	113-132	116-134	17
BM1706 [3]	16	80.6	234-256	232-254	232-252	4
BMS2639 [2]	18	57.0	156-180	166-186	166-178	2
BM6507 [1]	18	78.9	146-160	148-152	148-152	11
TGLA227 [2]	18	84.7	78-105	72	72	8
BMS2142 [1]	19	44.7	84-110	88-100	88-100	11
BM17132 [1]	19	58.6	79-103	79-89	75-89	3
BM3517 [3]	20	0.0	99-119	97-109	95-109	13
BM1225 [3]	20	8.0	230-263	242-276	242-272	4
BMS1128 [1]	20	33.9	84-100	92-95	92-94	6
AGLA29 [2]	20	50.6	144-160	133-142	133-137	9
BM4107 [3]	20	52.4	155-182	165-184	165-184	21
BMS1117 [1]	21	9.9	87-103	89-99	89-93	2
INRA194 [2]	22	21.8	145-165	143-159	143-157	18
PRL [2]	23	43.2	158-164	Null	Null	27
BMS2269 [1]	23	60.8	142-162	140-150	146-152	12
BM1905 [3]	23	64.3	170-198	170-184	172-182	4
BMS2270 [2]	24	21.2	77-95	63-67	63-65	11
BMS1862 [2]	24	32.8	149-199	142-170	142-170	2
BMS1743 [1]	24	40.7	137-160	146-152	146-153	17
BMS466 [1]	24	46.1	92-125	98-128	90-124	17
BMS130 [1]	25	12.3	148-238	148	148	15
HEL11 [12]	26	20.7	179-203	142-171	142-173	23
CSSM43 [4]	27	34.1	248-264	232-247	241-249	18
CSSM36 [4]	27	39.8	163-186	159-159	159-159	6
BM203 [3]	27	64.1	214-232	222-230	214-224	7

TABLE 4 CONTINUED

Locus	Chromosome	Position (cM)	DC Range	WBNP Range	YNP Range	Amplification Protocol
BMS1675 [1]	27	64.1	85-99	85-89	87-91	17
BMS510 [1]	28	22.1	82-108	91-95	91-95	3
BM4602 [1]	29	0.0	112-142	118-120	118-122	7
BMS1857 [2]	29	0.9	154-170	148-168	142-168	20
OCAM [8]	29	39.9	182-186	182-183	182-183	17
BMS903 [1]	X	12.1	92-129	78-102	78-96	6
MCM74 [13]	X	128.6	135-160	138-140	133-140	24
BMS911 [1]	X	136.2	90-108	96-108	96-102	24
BYM1 [16]	Y	-	255-257	242	NA	1

DC, domestic cattle; WBNP, Wood Buffalo National Park; YNP, Yellowstone National Park; Null, no amplification product was produced; NA, no genotypes were collected; [1], Stone *et al.* (1995); [2], Kappes *et al.* (1997); [3], Bishop *et al.* (1994); [4], Moore *et al.* (1994); [5], Barendse *et al.* (1994); [6], Shalom *et al.* (1994); [7], Buitkamp *et al.* (1995); [8], Moore *et al.* (1992); [9], Kemp *et al.* (1995); [10], Kemp *et al.* (1993); [11], Threadgill and Womack (1990); [12], Kaukinen and Varvio (1993); [13], Hulme *et al.* (1994); [14], Moore and Byrne (1992); [15], S. Davis (personal communication); [16], L. Skow (personal communication).

In order to identify microsatellite markers for which North American bison and domestic cattle did not share any native alleles in common, allelic distributions were determined from the Yellowstone National Park (YNP) and Wood Buffalo National Park (WBNP) populations of North American bison, and from domestic cattle representing the Angus, Hereford, Holstein, Shorthorn, and Longhorn breeds (Table 5). These five breeds of domestic cattle were chosen because they were the most important breeds in North America during the period when hybridization events were occurring (J. Sanders, personal communication), and were therefore the most likely breeds to have been used in hybridization experiments. The YNP and WBNP populations were chosen to represent native bison genetic variation because they are the only extant populations of North American bison to have continuously existed in the wild, and there is no evidence that they have a direct history of hybridization with domestic cattle (Coder 1975). In addition, both populations were screened with the domestic cattle specific mitochondrial marker described in the previous chapter, and no domestic cattle mtDNA haplotypes were identified in either of these populations. The YNP and WBNP populations represent the plains bison and wood bison subspecies respectively, and contain the vast majority of genetic variation identified in North American bison. For example, all but one of the 10 native mtDNA haplotypes identified in a survey of nine North American bison populations was found in at least one of these two populations (Polziehn *et al.* 1995). Similarly, the YNP and WBNP populations collectively contained 95.1% of all the allelic variation found in a survey of 11 microsatellites in 11 North American bison populations (Wilson and Strobeck 1999). Therefore, these two populations can be expected to adequately represent native allelic variation in North American bison.

Microsatellite markers with no alleles shared in common between domestic cattle and either the YNP or WBNP populations were considered to be completely diagnostic markers for examining domestic cattle introgression in North American bison. Therefore, a microsatellite marker was only considered uninformative if an allele was shared in common between domestic cattle, WBNP, and YNP. This approach was used to avoid potentially underestimating introgression due to the possibility that the YNP or WBNP population had themselves experienced introgression. In this way, alien alleles would not mistakenly be classified as native alleles unless they were present in both the YNP and WBNP populations. This situation is highly unlikely because there is no historical

TABLE 5

Domestic cattle and North American bison populations sampled

Species	Population	Abbreviation	Sample Size
Bos taurus	Angus	AN	10
(domestic cattle)	Hereford	HE	16
	Holstein	HO	13
	Shorthorn	SH	12
	Longhorn	TLH	13
Bison bison athabascae	Elk Island National Park, Canada	EIW	25
(wood bison)	Mackenzie Bison Sanctuary, Canada	MBS	36
	Wood Buffalo National Park, Canada	WBNP	23
Bison bison bison	Antelope Island State Park, UT	AI	32
(plains bison)	Caprock Canyon State Park, TX	JA	35
	Custer State Park, SD	CSP	39
	Elk Island National Park, Canada	EIP	25
	Finney State Game Refuge, KS	FSGR	32
	Fort Niobrara NWR, NE	FN	27
	Henry Mountains, UT	HM	21
	Maxwell State Game Refuge, KS	MSGR	40
	National Bison Range, MT	NBR	38
	Yellowstone National Park, WY	YNP	28
	Williams Ranch, TX	CW	11

NWR, National Wildlife Refuge.

connection between the YNP and WBNP populations (Coder 1975; Wilson and Strobeck 1999). Therefore, domestic cattle alleles introduced into one of these populations are not likely to be present in the other population.

In addition to domestic cattle from the five breeds described above, and North American bison from the WBNP and YNP populations, a total of 361 North American bison from 12 additional populations (Table 5) were genotyped for all of the diagnostic microsatellite markers identified. Completely diagnostic loci, with no alleles shared in common between domestic cattle and any of the North American bison, were assumed to be free of domestic cattle introgression. Alleles identified in North American bison that were shared by domestic cattle at one of these diagnostic loci were classified as potentially alien alleles. In order to determine if these shared alleles were due to introgression as opposed to allele size homoplasy or symplesiomorphy, genotypes were obtained from closely linked microsatellite markers (secondary markers), which are listed in Table 6. The hypothesis of introgression was rejected if individuals with a potentially alien allele at a diagnostic locus had alleles at closely linked microsatellite loci that were not shared by domestic cattle. The possibility of introgression was confirmed if an individual with a potentially alien allele at a diagnostic locus had an allele at a closely linked locus that was shared by domestic cattle, but not by North American bison from both the WBNP and YNP populations. Therefore, alien alleles were identified based on the association of shared alleles at diagnostic and secondary loci due to linkage disequilibrium. Individual North American bison were identified as having a domestic cattle chromosomal segment in a particular region of the genome when they possessed one or more of these alien alleles.

Examining the introgression of microsatellite markers closely linked to a particular gene of interest allows for the evaluation of hypotheses related to the introgression of functionally significant loci. For instance, during the period when hybridization between domestic cattle and North American bison was taking place, North American bison were experiencing a dramatic environmental change related to their being managed in captivity. These captive populations were largely managed by domestic cattle ranchers (Goodnight 1914; Coder 1975; Dary 1989). Thus, artificial selection for growth or conformation characteristics derived from a domestic cattle ancestry may have increased the frequency of introgression for chromosomal segments containing genes effecting these traits. Therefore, a microsatellite marker within the growth hormone factor-1 gene (PIT1 7B7) was evaluated for its utility in identifying introgression, because variation at this locus is known to effect growth and conformation characteristics in domesticated animals

TABLE 6

Secondary microsatellite markers used to identify domestic cattle chromosomal segments in North American bison

Locus	Chromosome	Position (cM)	Amplification Protocol	Reference
AGLA17	1	0.0	30	Kappes *et al.* 1997
BM6438	1	1.6	32	Bishop *et al.* 1994
TGLA49	1	1.9	32	Crawford *et al.* 1995
INRA117	1	8.4	38	Vaiman *et al.* 1994
BMS4017	1	34.8	39	Kappes *et al.* 1997
BM4307	1	35.2	29	Bishop *et al.* 1994
INRA119	1	68.7	30	Vaiman *et al.* 1994
BM6506	1	69.2	36	Bishop *et al.* 1994
BMS4008	1	71.7	33	Kappes *et al.* 1997
BMS4019	1	98.8	39	Kappes *et al.* 1997
BM6444	2	88.4	37	Bishop *et al.* 1994
RM103	5	28.6	40	Kossarek *et al.* 1995
AGLA293	5	31.2	31	Crawford *et al.* 1995
BMS1315	5	31.8	35	Stone *et al.* 1995
PRL2	23	43.2	37	*
RM185	23	45.1	34	Barendse *et al.* 1994
BM7233	23	49.1	36	Stone *et al.* 1995
ILSTS065	24	25.2	40	Kemp *et al.* 1995
BM1314	26	24.8	30	Bishop *et al.* 1994

*, The forward primer (5′-GGCTTGAGGTCAGAGAATTAAAGC-3′) and reverse primer (5′-CGTTGCATACAACCTCCTAAGT-3′) were designed from EMBL accession X16641 using MacVector 5.0 (International Biotechnologies Inc.).

(Yu *et al.* 1995; Renaville *et al.* 1997). A microsatellite within the kappa-casein gene (CSN3) was also examined, because variation at this locus has been shown to effect production characteristics in domestic cattle (Marzali and Ng-Kwai-Hang 1986), and positive diversifying selection is known to have impacted the evolution of this gene within the Bovidae (Ward *et al.* 1997). Microsatellites within the T-cell receptor beta gene (TCRB) and the natural resistance associated macrophage protein gene (NRAMP) were also examined, because these genes are believed to be involved in natural disease resistance and immune response (Feng 1996; Lewin 1996; Hackam 1998). Therefore, introgression may have differentially impacted these genes because North American bison were exposed to domestic cattle diseases at the same time that hybridization was occurring.

With the exception of BYM1 (the microsatellite on the Y chromosome), genotypes for all diagnostic and secondary markers were obtained for at least 98% of the domestic cattle and North American bison listed in Table 5. Due to the possibility that null alleles in domestic cattle could lead to an underestimate of introgression, exact tests of Hardy-Weinberg equilibrium were conducted using the program GENEPOP 3.1d (Raymond and Rousset 1995). When less than five alleles were present in a given population, the complete enumeration method was used to calculate an exact P-value. When at least five alleles were present in a given population, a Markov chain method with 10,000 dememorizations, 150 batches, and 40,000 iterations per batch was used to produce an unbiased estimate of the exact P-value. Allele frequencies were also calculated with the program GENEPOP 3.1d (Raymond and Rousset 1995).

Results

Of the 100 microsatellite markers examined in domestic cattle and North American bison from the WBNP and YNP populations, 21 were identified as being potentially diagnostic markers for identifying introgression. One of these markers (PIT1 7B7) had an allele (143) found in the Hereford breed of domestic cattle (allele frequency = 3.1%) that was also found in the YNP population (allele frequency = 1.8%) and was not found in the WBNP population. Therefore, this allele was classified as a potentially alien allele. However, the individuals in the YNP population with this potentially alien allele had alleles at a closely linked locus (BMS4017) that were not shared with any of the domestic cattle examined. Therefore, this shared allele is considered to be the result of allele size homoplasy or symplesiomorphy, and not introgression. Obviously, this means that PIT1

7B7 is not a completely informative marker. However, no alleles were shared in common between domestic cattle and North American bison from the YNP or WBNP populations at BMS4017. Therefore, this marker was found to be completely diagnostic for examining introgression.

Allele sharing at BMS827 was confined to an allele commonly found in both the WBNP and YNP populations, also being found in a single domestic cow from the Longhorn breed. Therefore, BMS827 was included as a diagnostic marker, because this degree of allele sharing would have a negligible effect on the ability to identify domestic cattle introgression at this locus. With the inclusion of BMS827, 22 diagnostic markers were identified. These markers were distributed across 12 of the 29 autosomes found in domestic cattle and North American bison, as well as the X and Y chromosomes. Multiple diagnostic markers were identified on five of the autosomes. However, these syntenic markers were separated by at least 25 cM.

The probability of detecting introgression in a given population is based on the frequency of alien alleles, the number of individuals sampled, and the number of diagnostic loci examined. The probabilities associated with detecting a 1% level of introgression were calculated as described in Davis *et al.* (1988) for each of the North American bison populations examined. Using the 20 autosomal diagnostic loci identified, the probability of detecting a 1% level of introgression was greater than 98% for all of the 13 publicly managed populations of North American bison examined and was 89% for the Williams Ranch population, where only 11 individuals were sampled.

The introgression of domestic cattle chromosomal segments was identified for seven of the 22 chromosomal regions containing diagnostic markers (Table 7). Three of these seven chromosomal segments were identified on chromosome 1, separated by roughly 25 cM. The other four chromosomal regions where introgression was identified are all on different chromosomes (Table 7). No introgression was observed for diagnostic loci on either the X or Y chromosomes. Domestic cattle alleles were identified in five of the 14 North American bison populations examined, with the frequency of introgression at a given locus ranging from 1.25% for region 3 in the Maxwell State Game Refuge (MSGR) population to 24.07% for region 2 in the Fort Niobrara National Wildlife Refuge (FN) population (Table 8). The Custer State Park (CSP) population had introgression in the greatest number of chromosomal regions (six out of 22, or 27.2%), and an even distribution of introgression across these chromosomal regions (range = 2.60 - 5.40%). In contrast, the Finney State Game Refuge population had the greatest frequency of

TABLE 7

Regions of the genome examined for evidence of introgression

	Locus	Chromosome	Position (cM)	DC Range	NAB Range	DC Alleles in NAB	North American Bison with Domestic Cattle Alleles
Region 1	AGLA17	1	0.0	212-217	213	217	CSP 17
	IFNAR15-2	1	0.7	159-161	167	161	CSP 17
	BM6438	1	1.6	257-268	253-270	257	CSP 17
	TGLA49	1	1.9	108-124	110	112	CSP 10,13,17
	INRA117	1	8.4	92-104	100-108	96	CSP 10,13,17
Region 2	PIT1 7B7	1	34.0	128-143	143-161	139; 139	CSP 19,30; FN 4*,6,7,10*,11,12,14,16,26,32,35
	BMS4017	1	34.8	148-158	145-165	148; 154	CSP 19,30; FN 4*,6,7,10,11,12,14,16,26,32,35
	BM4307	1	35.2	185-199	185-187	189; 197	CSP 19,30; FN 4*,6,7,10,11,12,14,16,26,32,35
Region 3	INRA119	1	68.7	130-138	119-130	132; 132; 132; 136	FSGR 2,6,7,10,12,13,17,25,26; MSGR 17; NBR 9,13,27,28,31; CSP 8,36
	BM7145	1	69.2	115-117	107-109	115; 115; 115; 115	FSGR 2,6,7*,10,12,13,17,25,26; MSGR 17; NBR 9,13,27,28,31; CSP 8
	BMS4008	1	71.7	152-179	158-164	166; 166; 166; 166	FSGR 2,6,7*,8,10,12,13,17,19,25,26; MSGR 17; NBR 8,9,13,27,28,31
Region 4	BMS4040	1	98.8	84-98	74 and 94		
	BMS4019	1	98.8	197-201	191-206		
Region 5	CSSM42	2	34.4	173-217	167-171		
Region 6	NRAMP	2	87.8	155-163	NO AMP.		
	BM6444	2	88.4	152-156	155-181		
Region 7	BMS827	4	24.7	91-105	89-91		
Region 8	BMS2809	4	72.0	144-171	140-142		
Region 9	TCRB	4	97.2	123-127	140-146		

TABLE 7 CONTINUED

	Locus	Chromosome	Position (cM)	DC Range	NAB Range	DC Alleles in NAB	North American Bison with Domestic Cattle Alleles
Region 10	BL23	5	28.6	242-256	234-236	246; 246	FSGR 4,25,30,32; MSGR 3,4,5,10,14,15,18,23,24,30
	AGLA293	5	32.0	218-250	218-220	228; 228	FSGR 4,25,30,32; MSGR 3,4,5,9,10,14,15,18,23,24,30
	BMS1315	5	32.5	135-147	134-148	135; 135	FSGR 4,25,30,32; MSGR 3,4,5,9,10,14,15,18,23,24,30
Region 11	RM500	5	55.6	122-132	120		
Region 12	SPS113	10	29.2	134-153	127-131		
Region 13	CSSM46	10	92.9	163-185	142-161		
Region 14	BM4513	14	62.5	135-162	128-130		
Region 15	TGLA227	18	84.7	78-105	72		
Region 16	AGLA29	20	50.6	144-160	133-142		
Region 17	PRL	23	43.2	158-164	NO AMP.	158	CSP 11,19,34
	PRL2	23	43.2	242-248	246	242	CSP 11,19,34
	RM185	23	45.1	90-108	92-94	100	CSP 11,19,34
	BM7233	23	49.1	100-124	103-121	113	CSP 11,19
Region 18	BMS2270	24	21.2	77-95	63-67	87	CSP 7,34
	ILSTS065	24	25.2	131-143	NO AMP.	143	CSP 7,34
Region 19	HEL11	26	20.7	179-203	142-175	187	CSP 7,24,29,30
	BM1314	26	24.8	143-168	137	158	CSP 24,29,30
Region 20	CSSM36	27	39.8	163-186	159		
Region 21	MCM74	X	128.6	135-160	133-140		

TABLE 7 CONTINUED

	Locus	Chromosome	Position (cM)	DC Range	NAB Range	DC Alleles in NAB North American Bison with Domestic Cattle Alleles
Region 22	BMY1	Y	-	255-257	242	

See Table 5 for population abbreviations. *, homozygous for a domestic cattle allele. NO AMP., native North American bison alleles did not amplify; DC, domestic cattle; NAB, North American bison.

TABLE 8

Frequency (%) of domestic cattle chromosomal segments in North American bison populations

	CSP	FN	FSGR	MSGR	NBR
Region 1	3.80				
Region 2	2.60	24.07			
Region 3	2.60		18.75	1.25	7.89
Region 10			6.25	13.75	
Region 17	3.80				
Region 18	2.60				
Region 19	5.40				
Overall	0.93	1.09	1.14	0.68	0.36

See Table 5 for population abbreviations.

introgression averaged over all 22 regions (1.14%), but this was the result of introgression in only two chromosomal regions (Table 8).

Allele frequencies for each of the microsatellite markers listed in Table 7 were calculated (Table 9). Of the 25 different domestic cattle alleles identified in North American bison (Table 7), 16 (64%) were also the most common allele found in the sample of 64 domestic cattle, and 20 (80%) of these alleles were the most common allele found in at least one of the five domestic cattle breeds. This association is predicted by the hypothesis that these alleles are present in North American bison as a result of introgression. However, this association would not be predicted by hypotheses involving symplesiomorphy or allele size homoplasy.

The 22 chromosomal regions in which diagnostic markers were identified include the growth hormone factor-1 gene (PIT1 7B7), the natural resistance associated macrophage protein gene (NRAMP), and the T-cell receptor beta gene (TCRB). The microsatellite, CSN3, was classified as uninformative because it had alleles shared in common between domestic cattle, the WBNP population, and the YNP population. No introgression was observed at the NRAMP or TCRB loci, however this was consistent with the generally low level of introgression observed across all 22 of the chromosomal regions examined. Domestic cattle chromosomal segments were identified at the PIT1 7B7 marker and two closely linked loci in region 2 (Table 7). The frequency of introgression in this region was 2.6% in the CSP population and 24.07% in the FN population. The domestic cattle haplotypes composed of the alleles at the PIT1 7B7, BMS4017, and BM4307 microsatellite markers in region 2, were different in the CSP (139:148:189) and FN (139:154:197) populations (Table 7).

In order to examine the possibility that null alleles were present at some of the microsatellite markers examined, tests of Hardy-Weinberg equilibrium were conducted for each of the microsatellite markers listed in Table 7. Separate tests were conducted for each of the 14 North American bison populations and the five domestic cattle breeds. A total of 21 significant ($P < 0.05$) deviations from Hardy-Weinberg equilibrium were detected in the 443 tests performed. Given this number of tests, 22 deviations are expected as a result of random chance. With only one exception, no more than two significant ($P < 0.05$) deviations were detected for the same marker. However, five of the North American bison populations and one of the domestic cattle breeds had significant ($P < 0.05$) deviations from Hardy-Weinberg equilibrium at the HEL11 locus. This locus displayed extreme heterogeneity in the amplification intensity of different alleles in North American

TABLE 9

Allele frequencies for diagnostic and secondary microsatellite markers

AGLA17																		
Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
212															0.100	0.313	0.308	0.125	0.077
213	1.000	0.987	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000					
214																			0.077
217		**0.013**													0.900	0.688	0 692	0.875	0.846

IFNAR15-2																		
Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
159															0.100		0.038	0.250	0.038
161		**0.013**													0.900	1.000	0.962	0.750	0.962
167	1.000	0.987	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000					

BM6438																			
Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
253	0.250	0.632	0.818	0.540	0.200	0.565	0.578	0.667	0.429	0.431	0.692	0.671	0.304	0.643					
257		**0.013**													1.000	0.969	0.538	0.667	1.000
259																	0.231		
264	0.156	0.039					0.063												
266		0.039									0.051	0.118		0.018					
268	0.594	0.263	0.182	0.280	0.800	0.435	0.359	0.333	0.571	0.500	0.256	0.184	0.543	0.268		0.031	0.231	0.333	
270		0.013		0.180						0.069		0.026	0.152	0.071					

TGLA49																			
Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
108															0.150				0.038
110	1.000	0.962	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.050	0.438	0.038		
112		**0.038**													0.050		0.346	0.125	
115															0.750	0.563	0.308	0.875	0.577
117																			0.346
124																	0.308		0.038

TABLE 9 CONTINUED

INRA117

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
92																			0.077
96		**0.039**													1.000	1.000	1.000	1.000	0.808
98																			0.038
100				0.140															
102	0.141			0.020	0.340		0.016	0.300		0.333		0.053	0.109	0.089					0.038
104	0.563	0.342	0.818	0.580	0.100	0.125	0.172	0.400	0.143	0.181	0.423	0.618	0.370	0.518					0.038
106	0.031	0.592	0.182	0.260	0.480	0.625	0.813	0.225	0.529	0.375	0.462	0.289	0.391	0.250					
108	0.266	0.026			0.080	0.250		0.075	0.329	0.111	0.115	0.039	0.130	0.143					

PIT1 7B7

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
128															0.350	0.125	0.269	0.500	0.167
132																0.031			
133															0.050	0.094	0.077		0.056
135																0.094			
137															0.050		0.077	0.042	
139		**0.026**				**0.222**									0.500	0.625	0.577	0.417	0.778
141															0.050			0.042	
143	0.094	0.038				0.093	0.047	0.024	0.114		0.038	0.039		0.018		0.031			
145	0.813	0.538	0.727	0.500	0.320	0.333	0.250	0.619	0.414	0.222	0.287	0.276	0.239	0.375					
147		0.090		0.040															
150				0.040	0.260					0.292		0.053	0.283	0.179					
155	0.031	0.244	0.045	0.220	0.040	0.056	0.141	0.214		0.014	0.038	0.158	0.087	0.179					
157	0.016		0.227		0.180	0.019	0.031	0.071		0.167	0.125	0.395	0.174	0.250					
159	0.047	0.064		0.200	0.200	0.278	0.531	0.071	0.171	0.306	0.512	0.066	0.217						
161									0.300			0.013							

TABLE 9 CONTINUED

BMS4017

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
145														0.143					
148		**0.027**													0.450	0.133	0.577	0.625	0.654
153		0.068	0.136	0.040				0.095		0.014	0.013		0.022						
154						**0.204**									0.500	0.200	0.115	0.250	0.231
155	0.016	0.514	0.500	0.320	0.080	0.500	0.891	0.167	0.629	0.625	0.563	0.553	0.478	0.429					
156																0.500	0.038	0.125	0.115
157					0.260				0.343	0.125		0.039	0.065						
158															0.050	0.167	0.269		
159	0.938	0.122	0.273		0.500	0.130		0.071		0.125	0.262	0.079	0.196	0.232					
161	0.016	0.122				0.019		0.048			0.100			0.018					
163	0.031	0.149	0.091	0.160	0.160	0.148	0.109	0.405	0.029	0.111	0.050	0.092	0.152	0.161					
165				0.480				0.214			0.013	0.237	0.087	0.018					

BM4307

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
185															0.111				
187	0.984	0.756	0.545	0.958	0.580	0.778	0.844	0.929	0.457	0.722	0.600	0.855	0.674	1.000	0.222	0.125	0.167	0.091	
189	0.016	0.218	0.455	0.042	0.420	0.019	0.156	0.071	0.543	0.278	0.400	0.145	0.326			0.031			
191		**0.026**													0.111	0.375	0.542	0.545	0.192
195																0.063	0.042	0.091	0.154
197						**0.204**									0.556	0.406	0.250	0.227	0.385
199																		0.045	0.269

TABLE 9 CONTINUED

INRA119

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
119		0.026																	
122		0.205	0.455	0.040		0.185	0.094	0.405	0.357	0.042	0.138		0.022	0.268					
124	0.063	0.551	0.455	0.420	0.880	0.778	0.531	0.524	0.571	0.722	0.625	0.500	0.783	0.375					
126			0.091	0.200	0.020		0.016			0.056	0.013	0.066	0.022	0.018					
128	0.938	0.192		0.340	0.060	0.037	0.219	0.071	0.071	0.153	0.213	0.368	0.174	0.339					
130					0.040					0.028					0.222	0.344	0.038	0.042	0.115
132							**0.141**				**0.013**	**0.066**			0.222	0.156	0.615	0.292	0.269
134															0.167	0.438		0.208	0.423
136		**0.026**													0.389	0.063	0.346	0.458	0.154
138																			0.038

BM7145

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
107	0.953	0.718	1.000	0.708	0.780	0.889	0.844	0.786	1.000	0.764	0.775	0.934	0.804	0.786					
109	0.047	0.269		0.292	0.220	0.111		0.214		0.236	0.213		0.196	0.214					
115		**0.013**					**0.156**				**0.013**	**0.066**			0.900	0.969	0.654	0.958	0.885
117															0.100	0.031	0.346	0.042	0.115

TABLE 9 CONTINUED

BMS4008

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
152																			0.154
156															0.444	0.188	0.231	0.208	0.423
158		0.013											0.022			0.281		0.125	
160	0.274	0.667	0.682	0.540	0.840	0.907	0.453	0.905	0.471	0.542	0.762	0.395	0.565	0.696		0.125		0.167	0.192
162	0.726	0.321	0.318	0.440	0.160	0.093	0.359	0.095	0.529	0.444	0.225	0.526	0.413	0.125					
164				0.020						0.014				0.179		0.031	0.077		
166							**0.188**				**0.013**	**0.079**			0.056	0.344	0.385	0.167	
168																	0.077		
172															0.056				
174															0.056				0.038
177																	0.192	0.167	0.077
179															0.389	0.031	0.038	0.167	0.115

BMS4040

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
74	1.000	0.987	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000					
84																0.188	0.038	0.083	0.154
86															0.050				0.038
94		0.013																	
96															0.900	0.656	0.962	0.917	0.808
97															0.050	0.031			
98																0.125			

TABLE 9 CONTINUED

BMS4019

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
191													0.043						
197											0.013				0.500	1.000	0.731	0.375	0.885
199		0.111		0.120			0.156	0.190		0.014	0.138		0.043	0.089	0.500		0.269	0.500	0.077
201						0.042					0.087							0.125	0.038
203	1.000	0.889	1.000	0.880	1.000	0.958	0.844	0.810	1.000	0.986	0.762	1.000	0.870	0.911					
206													0.043						

CSSM42

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
167	0.500	0.635	0.955	0.740	0.646	0.800	0.703	0.714	0.671	0.914	0.603	0.566	0.804	0.589					
169	0.453	0.081	0.045	0.120	0.229			0.071		0.086	0.051	0.026	0.109	0.054					
171	0.047	0.284		0.140	0.125	0.200	0.297	0.214	0.329		0.346	0.408	0.087	0.357					
173																0.094		0.333	
175															0.150			0.042	
177																	0.038	0.042	0.038
179															0.300	0.125	0.231	0.375	0.346
181																0.031		0.042	
193																	0.077		
205																0.031	0.077	0.042	0.038
207																			0.038
209																		0.042	
211																0.031			0.038
213															0.550	0.688	0.154	0.083	0.462
217																	0.423		0.038

NRAMP1

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
Null	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000					
155															0.900	1.000	1.000	1.000	0.846
157															0.100				0.115
163																			0.038

TABLE 9 CONTINUED

BM6444

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
152															0.400	0.679	0.385	0.375	
154															0.600	0.286	0.577	0.375	0.692
155												0.013							
156																0.036	0.038	0.250	0.308
158	0.328	0.295	0.091	0.520	0.080	0.520	0.313	0.048	1.000	0.014	0.605	0.171	0.068	0.393					
160			0.091			0.120													
164	0.203	0.115	0.136	0.100		0.220	0.125	0.119		0.194	0.105	0.329	0.182	0.250					
166	0.375	0.128	0.091	0.100	0.240		0.438	0.119		0.375	0.066	0.184	0.227	0.071					
172						0.040													
174	0.047	0.269	0.182	0.280	0.340	0.100				0.097	0.053	0.224	0.114	0.089					
175								0.024											
176	0.047	0.167	0.409		0.340		0.078	0.690		0.319	0.171	0.079	0.409	0.196					
181		0.026					0.047												

BMS827

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
89	0.984	0.795	0.955	0.900	0.520	0.860	0.953	0.548	0.686	0.653	0.800	0.855	0.739	0.750					
91	0.016	0.205	0.045	0.100	0.480	0.140	0.047	0.452	0.314	0.347	0.200	0.145	0.261	0.250					0.038
93															0.100				
97															0.200	0.438	0.115	0.125	0.423
99															0.300	0.031	0.500	0.583	0.077
101															0.250	0.031	0.308	0.125	0.231
103															0.150	0.219	0.077	0.167	0.231
105																0.281			

TABLE 9 CONTINUED

BMS2809

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
140	0.177		0.136	0.021	0.104	0.148				0.043			0.196						
141						0.056			0.014			0.013							
142	0.823	1.000	0.864	0.979	0.896	0.796	1.000	1.000	0.986	0.957	1.000	0.987	0.804	1.000					
144																			0.115
148																			0.038
159															0.100	0.031	0.542	0.125	0.346
161																0.125	0.167		0.077
163															0.250			0.167	
165																0.063		0.042	0.346
167															0.100		0.042	0.125	
169															0.550	0.750	0.208	0.542	0.038
171																0.031	0.042		0.038

TCRB

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
123																		0.042	
125															1.000	1.000	0.923	0.958	1.000
127																	0.077		
140				0.060									0.043						
144	0.266	0.230		0.520	0.300	0.519	0.219	0.143	0.371	0.500	0.205	0.197	0.478	0.125					
146	0.734	0.770	1.000	0.420	0.700	0.481	0.781	0.857	0.629	0.500	0.795	0.803	0.478	0.875					

TABLE 9 CONTINUED

BL23

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
234	1.000	1.000	1.000	1.000	1.000	1.000	0.938	1.000	1.000	0.944	0.872	1.000	0.935	1.000					
236										0.056			0.065						
242																0.094		0.083	
244															0.056	0.063	0.038		0.125
246							**0.063**				**0.128**				0.667	0.406	0.308	0.583	0.208
248															0.278	0.344	0.577	0.333	0.583
250																0.094	0.038		0.083
256																	0.038		

AGLA293

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
218	1.000	0.962	1.000	0.960	1.000	1.000	0.938	1.000	1.000	1.000	0.837	0.987	1.000	1.000		0.406			
220		0.038		0.040							0.025	0.013					0.038		
222															0.250		0.038	0.042	
225																			0.250
226																			0.083
228							**0.063**				**0.138**				0.750	0.563	0.846	0.458	0.125
230																		0.292	0.125
232																		0.125	0.042
236																			0.083
239																0.031	0.077	0.083	0.042
246																			0.083
248																			0.125
250																			0.042

TABLE 9 CONTINUED

BMS1315

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
134	0.375	0.595	0.636	0.780	0.146	0.500	0.274	0.286	0.186	0.264	0.387	0.553	0.348	0.536					
135							**0.065**				**0.138**				0.600	0.333	0.615	0.750	0.308
136		0.081	0.364	0.060			0.323		0.814	0.028	0.100	0.145		0.196					
137															0.150	0.133		0.042	
139															0.250	0.267	0.231	0.083	0.154
140	0.516	0.108		0.080	0.583			0.190		0.222	0.138	0.105	0.217	0.196					
143																0.267	0.038	0.083	0.538
144					0.250					0.375			0.391						
146	0.109	0.216		0.080	0.021	0.389	0.242	0.524		0.111	0.213	0.197	0.043	0.071					
147																	0.115	0.042	
148						0.111	0.097				0.025								

RM500

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
120	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000					
122																			0.111
124															0.100	0.344	0.154	0.125	0.389
126																	0.038		
128															0.200	0.063	0.192	0.125	0.500
130															0.650	0.594	0.577	0.708	
132															0.050		0.038	0.042	

TABLE 9 CONTINUED

SPS113

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
127												0.132							
129	0.750	0.705	0.409	0.540	0.760	0.667	0.258	0.048	1.000	0.556	0.575	0.487	0.478	0.571					
131	0.250	0.295	0.591	0.460	0.240	0.333	0.742	0.952		0.444	0.425	0.382	0.522	0.429					
134															0.050				0.115
136															0.100	0.333		0.167	
138															0.200		0.154	0.125	0.192
140																0.033		0.083	
142																			0.038
144																	0.192		0.192
146															0.100				0.192
148															0.050	0.100	0.577	0.250	
150															0.500	0.533	0.077	0.292	0.269
153																		0.083	

TABLE 9 CONTINUED

CSSM46

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
142											0.013								
143	0.031	0.167		0.040		0.167	0.367	0.150		0.014	0.368			0.339					
154	0.969	0.625	1.000	0.960	1.000	0.833	0.633	0.850	1.000	0.986	0.618	0.932	1.000	0.661					
158												0.068							
161		0.208																	
163															0.150		0.038	0.042	
164																			0.038
166																			0.115
167																	0.115		
168															0.100				
169																0.188	0.077	0.042	
170																		0.375	
171															0.350	0.125	0.077	0.167	0.269
172																	0.077		0.077
173																		0.083	0.192
174															0.200	0.188	0.115	0.125	0.154
175																0.063	0.038	0.042	
176															0.050	0.375	0.115	0.083	0.038
181																	0.038		
185															0.150	0.063	0.308	0.042	0.115

TABLE 9 CONTINUED

BM4513

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
128	0.891	0.679	0.818	1.000	1.000	0.981	0.953	0.857	1.000	1.000	0.936	1.000	1.000	0.839					
130	0.109	0.321	0.182			0.019	0.047	0.143			0.064			0.161					
135																0.031	0.077		0.038
137																			0.038
139															0.300	0.313	0.038	0.167	0.231
141															0.150	0.094	0.038		0.308
143															0.050	0.156	0.385	0.375	0.077
145															0.400	0.156	0.154	0.125	0.231
147															0.050		0.115	0.250	0.038
150																0.156		0.042	
156															0.050		0.154		
158																0.094			0.038
160																	0.038		
162																		0.042	

TGLA227

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
72	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000					
78																			0.167
82															0.150		0.038	0.167	0.222
84																0.313		0.042	
89															0.050	0.031			0.056
91															0.400	0.250	0.192	0.667	0.111
93															0.150	0.281	0.192	0.042	0.056
95															0.100	0.094	0.038	0.083	0.111
97																	0.038		
100															0.150	0.031	0.423		0.278
105																	0.077		

TABLE 9 CONTINUED

AGLA29

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
133	0.016	0.184		0.140	0.120			0.238		0.028	0.154	0.197	0.043	0.107					
135				0.080									0.043						
137	0.984	0.803	1.000	0.780	0.800	1.000	1.000	0.762	1.000	0.792	0.846	0.803	0.804	0.893					
139		0.013																	
142					0.080					0.181			0.109						
144																			0.462
146															0.100	0.125	0.385	0.042	0.038
148																0.281		0.125	0.038
150															0.300	0.313	0.115	0.167	0.154
152															0.050	0.063			
154															0.150	0.063	0.115	0.250	0.269
156																0.094			
158															0.100	0.063	0.385	0.292	
160															0.300			0.125	0.038

PRL

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
Null	1.000	0.962	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000					
158		**0.038**													0.167	0.031	0.115	0.125	0.308
162															0.778	0.969	0.885	0.875	0.692
164															0.056				

PRL2

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
242		**0.041**													0.150	0.031	0.115	0.125	0.308
246	1.000	0.959	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.800	0.969	0.885	0.875	0.692
248															0.050				

TABLE 9 CONTINUED

RM185

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
90																			0.077
92	1.000	0.962	1.000	0.960	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.050				
94				0.040												0.036	0.192	0.083	0.038
96															0.200	0.036		0.333	
98															0.100		0.115		
100		**0.038**													0.150	0.214	0.115	0.042	0.115
102															0.300	0.607	0.346	0.208	0.308
104															0.100	0.036	0.192	0.125	0.038
106															0.100	0.071	0.038	0.167	0.423
108																		0.042	

BM7233

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
100															0.250	0.250	0.308	0.167	0.423
103	0.297	0.645	1.000	0.780	0.840	0.917	1.000	0.952	0.957	0.797	0.900	0.684	0.783	0.750					0.038
104																	0.115	0.125	0.154
105		0.118				0.063		0.048	0.043			0.092	0.022	0.250					
106															0.050			0.083	
108																			0.038
113		**0.026**																	0.077
114		0.158		0.020															
115															0.400	0.750	0.115	0.542	0.077
116	0.703			0.120		0.021				0.031	0.038	0.184	0.043						
117															0.050		0.346		0.154
118		0.026		0.080	0.160					0.172			0.152						
119															0.200		0.077		0.038
121		0.026									0.063	0.039							
122															0.050				
124																	0.038	0.083	

TABLE 9 CONTINUED

BMS2270

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
63	0.258	0.474	0.636	0.708	0.660	0.296	0.281	0.786	0.814	0.514	0.600	0.697	0.674	0.518					
65	0.742	0.346	0.364	0.292	0.220	0.704	0.578	0.214		0.292	0.287	0.289	0.196	0.482					
67		0.154			0.120		0.141		0.186	0.194	0.112	0.013	0.130						
77																0.033	0.038		
79																0.233		0.333	0.115
81																0.100	0.038	0.125	0.346
83																			0.077
85															0.100		0.308		
87		**0.026**													0.100	0.267	0.115	0.125	0.269
89															0.200		0.192	0.208	0.038
91															0.050			0.042	0.077
93																0.300			
95															0.550	0.067	0.308	0.167	0.077

ILSTS065

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
Null	1.000	0.974	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000					
131		**0.026**													0.500	0.429	0.385	0.364	0.167
133																		0.045	0.167
135															0.222	0.214	0.077	0.091	0.125
137															0.167	0.321	0.038	0.409	0.042
139																			0.083
141																0.036	0.500	0.091	0.083
143															0.111				0.333

TABLE 9 CONTINUED

HEL11

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
142		0.122	0.091	0.042		0.130					0.111	0.013	0.022	0.107					
148		0.041		0.083	0.480			0.071		0.514		0.039	0.304	0.232					
153		0.041		0.208			0.031			0.028	0.056			0.054					
155	0.906	0.324		0.125	0.260	0.130	0.734		0.043	0.069	0.417	0.171	0.087	0.071					
156		0.027		0.063	0.080	0.333	0.156			0.208		0.105	0.239	0.125					
157					0.060						0.014								
159		0.068	0.364	0.021		0.074		0.095					0.174	0.107					
160	0.016																		
161	0.016	0.270		0.313		0.259	0.031	0.833	0.643	0.083	0.097	0.026	0.043	0.232					
163		0.054							0.314	0.014	0.069	0.066	0.043	0.054					
165	0.016												0.022						
167			0.045							0.056			0.043						
171				0.042	0.040					0.014	0.028	0.276	0.022						
173	0.047		0.500	0.104	0.080	0.019	0.047				0.181	0.303		0.018					
175						0.056				0.014	0.028								
179															0.111	0.375	0.462	0.250	0.077
183															0.167			0.250	0.077
185															0.111	0.125	0.038	0.083	
187		**0.054**													0.222	0.188	0.231	0.042	0.385
189															0.222	0.281	0.192	0.042	0.154
191															0.167	0.031	0.038	0.333	0.192
195																			0.038
197																			0.038
203																	0.038		0.038

TABLE 9 CONTINUED

BM1314

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
137	1.000	0.959	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000					
143																			0.038
145																			0.038
148															0.056	0.250			
154															0.056	0.313	0.038		
156															0.667	0.344	0.615	0.333	0.231
158		**0.041**													0.167		0.346	0.583	0.423
160															0.056				0.192
164																0.063			0.038
166																		0.083	
168																0.031			0.038

CSSM36

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
159	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000					
163															0.100	0.094	0.167	0.292	0.346
168																	0.083		0.038
170																0.031	0.042		
172															0.050	0.125	0.042	0.125	
174															0.050	0.094	0.292		0.423
176															0.200			0.083	0.077
178																			0.077
180															0.550	0.438	0.333	0.167	0.038
182															0.050	0.219		0.333	
186																	0.042		

TABLE 9 CONTINUED

MCM74

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
133	0.963	0.185	0.474	0.306				0.029		0.020		0.286		0.054					
135																			0.192
138	0.037	0.796	0.263	0.528	0.738	1.000	0.780	0.629	0.123	0.667	0.981	0.607	0.839	0.839					
140		0.019	0.263	0.167	0.262		0.220	0.343	0.877	0.314	0.019	0.107	0.161	0.107					
143															0.600	0.438	0.429	0.429	0.077
145																			0.192
147																	0.143	0.071	
149																	0.048	0.143	0.192
151															0.100	0.250		0.071	
153															0.300	0.313	0.381	0.286	0.269
157																			0.038
160																			0.038

BMY1

Allele Size	AI	CSP	CW	EIP	EIW	FN	FSGR	HM	JA	MBS	MSGR	NBR	WBNP	YNP	AN	HE	HO	SH	TLH
242	1.000	1.000	1.000	1.000	1.000	NA	1.000	1.000	1.000	1.000	1.000	1.000	1.000	NA					
255																		0.400	
257															1.000	1.000	1.000	0.600	1.000

See Table 5 for population abbreviations. Values shown in bold are frequencies for domestic cattle alleles found in North American bison populations. NA, no genotypes were collected.

bison, which probably accounts for the large number of significant deviations. While, null alleles may account for the pattern seen at HEL11, it is unlikely that they would cause introgression to be underestimated, because domestic cattle chromosomal segments would likely have been detected at BM1314, which is only 4.1 cM away from HEL11 and had no significant deviations from Hardy-Weinberg equilibrium.

Discussion

Mitochondrial markers provide an excellent source of information for the identification of populations with a hybrid ancestry. However, the joint consideration of information from mtDNA and nuclear markers is required to identify all populations which have experienced introgressive hybridization, and to completely characterize the impact that hybridization events have had on a species or population. By incorporating information on linkage relationships, large numbers of microsatellite markers can be used in studies of introgressive hybridization, and can greatly increase the power of such studies.

The use of diagnostic microsatellite markers from 22 different chromosomal regions allowed for the identification of domestic cattle nuclear introgression in four of the seven North American bison populations where mtDNA introgression had already been documented. In addition, nuclear introgression was identified in the FN population, where no mtDNA introgression has been observed. The 22 microsatellite markers also provided a method of assessing the probability that populations for which nuclear introgression was not identified, actually contained domestic cattle alleles at a frequency of 1% or greater across the genome. Therefore, by combining the data from mitochondrial and nuclear markers, all of the North American bison populations with appreciable levels of introgression were identified.

The combination of information from nuclear and mitochondrial markers also provides information about the nature of the hybridization events themselves. For instance, no nuclear introgression was identified in three populations where domestic cattle mtDNA introgression was documented in the previous chapter, and the absence of Y chromosome introgression indicates that there may have been a bias in the direction of hybridization. The absence of Y chromosome introgression is at least partially explained by the infertility of first generation hybrid (F1) males. However, matings between domestic cattle bulls and F1 or backcross generation hybrid females could produce fertile male offspring (Boyd 1914) which would obviously carry a domestic cattle Y

chromosome. Several historical reports, describing attempts to hybridize domestic cattle and North American bison in captivity, have indicated that male domestic cattle were largely unwilling to breed North American bison females, while male North American bison would readily breed domestic cattle females (Boyd 1908, 1914; Dary 1989). Therefore, the absence of domestic cattle Y chromosome introgression may be the result of a behavioral constraint on the direction of hybridization. Female mediated introgression, when combined with the sterility of F1 hybrid males, could quickly result in a population with alien mtDNA in the absence of significant nuclear introgression (Aubert and Solignac 1990), and could explain the differential introgression of domestic cattle mtDNA haplotypes in North American bison populations.

Information about the dynamics of hybridization and introgression events can also be obtained from the distribution of introgression across different chromosomal regions. For instance, nuclear introgression in the CSP population involved many different regions of the genome (6 of 22, or 27.3%), with a relatively low frequency of introgression in each region (range = 2.6 - 5.4%). In addition, domestic cattle mitochondrial or nuclear alleles were identified in 14 of the 39 (35.9%) individuals examined, with no individual having domestic cattle alleles at more than two nuclear regions. This population is known to have a direct history of hybridization with domestic cattle (Coder 1975), and the homogenous distribution of domestic cattle alleles throughout this population and across the genome is indicative of a population which has reached equilibrium with respect to introgression.

In contrast to the homogenous distribution of introgression observed in the CSP population, a heterogeneous distribution was observed in the other four populations where nuclear introgression was identified. While this pattern could be indicative of differential selection, it is more likely the result of secondary introductions of domestic cattle alleles into these populations due to recent transfers from other populations with domestic cattle introgression. For example, nuclear introgression in the National Bison Range (NBR) population was limited to region 3, where NBR had the same domestic cattle haplotype as that seen in the Finney State Game Refuge (FSGR) and Maxwell State Game Refuge (MSGR) populations (Table 7). Park records (D. Wiseman, personal communication) and the mtDNA haplotype data presented in Table 3 were used to determine that the North American bison with domestic cattle mtDNA haplotypes present in the National Bison Range (NBR) population are the result of recent transfers from the Maxwell State Game Refuge (MSGR) population. It is likely that this transfer is also responsible for the

presence of individuals with domestic cattle nuclear introgression in NBR. However, this could not be confirmed with park records. In addition, it is not clear whether the FSGR and MSGR populations have a direct history of hybridization or have had domestic cattle genes introduced via secondary introductions of late generation hybrid backcross individuals. However, the domestic cattle haplotypes identified in region 3 and region 10 further support the inference that there is a common source of introgression for these two populations, as described in the previous chapter.

In addition to producing information on the geographical and genomic distributions of introgression, the incorporation of information from linkage maps allows additional aspects of introgressive hybridization to be characterized. For instance, the possibility that North American bison could have experienced changes in selective pressures associated with captivity, led to hypotheses about the selective retention of introduced cattle variation at loci known to effect growth, conformation and immune response. By identifying diagnostic microsatellite markers closely linked to, or even within such genes, introgression at these functionally significant loci could be documented. No introgression was identified at either the TCRB or NRAMP loci in any of the populations examined, obviously eliminating the possibility that domestic cattle variation was selectively retained at these loci. However, the frequency of introgression within the region containing the growth hormone factor-1 gene was higher in the FN population than at any other region in any population examined (Table 8), and included two individuals which were homozygous for a domestic cattle allele. This could be the result of inadvertent artificial selection for North American bison with size and conformation characteristics derived from a domestic cattle ancestry. However, it could also be the result of genetic drift in small populations. This later explanation is especially likely for populations in which secondary introductions of domestic cattle genes have been made via transfers from populations with a direct hybrid ancestry. For instance, domestic cattle introgression was also identified for the region encompassing the growth hormone factor-1 gene in the CSP population, from which eight bulls were introduced into FN during the 1930's. Different domestic cattle haplotypes were identified in region 2 in these two populations, but this could be the result of limited sampling within the CSP population.

Uncertainty about the origin of the domestic cattle alleles found in the FN population makes it difficult to derive conclusions regarding the possibility that selective retention of domestic cattle variation has shaped the current genomic distribution of introgression in this population. However, CSP has a documented history of direct

hybridization with domestic cattle. The fact that introgression of the region containing the growth hormone factor-1 gene was limited to a frequency of 2.6% in this population is inconsistent with the hypothesis that positive selection has caused domestic cattle variation to be retained at this locus in North American bison. Obviously, this does not rule out the possibility that positive selection has influenced introgression at other loci effecting these traits.

Using a disequilibrium mapping approach, the introgression of chromosomal segments as large as 8 cM in length (Table 7) was identified in North American bison. With the examination of greater numbers of genomic regions, the differential introgression of whole chromosomes or large chromosome segments could be documented. For example, seven of the 12 (58.3%) instances where nuclear introgression was identified in North American bison (Table 7), involved regions of chromosome 1, even though chromosome 1 contained only 4 of the 22 (18.2%) regions examined. Although, it is unlikely that introgression in the different populations examined represents independent hybridization events, it is possible that chromosome 1 is particularly permissive to introgression, perhaps indicating a lack of species incompatibility genes on this chromosome. Obviously, too few regions have been examined to test this hypothesis, but with the examination of a larger number of genomic regions, instances of differential introgression in certain regions of the genome could be documented. This would obviously be useful for understanding the nature of species boundaries and for identifying the genes responsible for species incompatibility.

The ability to identify even low levels of introgression in North American bison, stemming from hybridization events that occurred over 100 years ago, demonstrates the power of a disequilibrium mapping approach for studies of introgression. With the increasing information derived from genome mapping efforts, the addition of information on the linkage relationships between molecular markers can be used to identify introgression in a broad range of taxa. Much of the disagreement over the significance of hybridization in evolution is based on differences in the perceived frequency of hybridization and introgression (Anderson 1948; Mayr 1963; Harrison 1993; Dowling and Secor 1997). Therefore, the enhanced ability to identify introgression provided by the use of linked molecular markers could enable a significant advance in the understanding of the evolutionary significance of introgressive hybridization.

A TEST OF ALTERNATIVE THEORIES REGARDING THE EVOLUTIONARY SIGNIFICANCE OF INTROGRESSIVE HYBRIDIZATION

Synopsis

The predictions from alternative theories regarding the evolutionary significance of hybridization in evolution were tested using a simulation model based on the history of hybridization between North American bison and domestic cattle. Observed levels of introgression were found to be consistent with neutral expectations for a mitochondrial marker, a marker on the X chromosome, and 20 autosomal markers. These findings indicate that exogenous selection has not played a major role in the introgression of domestic cattle genes into North American bison populations, and are inconsistent with predictions based on a view of introgressive hybridization as a creative evolutionary force. However, simulations based on the history of hybridization in the Custer State Park population of North American bison indicate that hybridization with domestic cattle would have slightly decreased the probability of population extinction, and would have resulted in a slightly greater fraction of native genetic variation being preserved. These differences are expected to be greater for populations with slower rates of intrinsic growth. While there is no indication that hybridization played a significant role in the recovery of North American bison from a dramatic demographic decline, these results indicate that introgressive hybridization could act as a positive evolutionary force, even when selection against hybrid individuals is strong.

Introduction

Introgressive hybridization occurs when a cross between representatives of two genetically distinct populations produces offspring that backcross to one or both parental populations, resulting in an exchange of genes between these populations (Anderson and Hubricht 1938). Many authors have argued that introgressive hybridization is a creative evolutionary force, which results in the transfer of large amounts of novel genetic variation between participating taxa, and can lead to adaptive evolution through the transfer of adaptations between taxa, or the creation of novel genetic combinations which are favored in certain environments (Anderson 1948; Anderson and Stebbins 1954; Lewontin and Birch 1966; Grant and Grant 1992; Arnold 1997). Others have argued that hybridization

is maladaptive, or has only transient effects, and that successful introgression will be limited by environment-independent selection (endogenous selection) against hybrids, due to the breakdown of co-adapted gene complexes and the presence of genetic and cytological incompatibilities that have arisen between the parent taxa in the course of divergent evolution (Dobzhansky 1937, 1940; Mayr 1942, 1963). In large part, these differences result from different views about the relative importance of environment dependent selection (exogenous selection) in shaping the outcome of hybridization events. For instance, under a negative view of hybridization, endogenous selection is assumed to be of greater importance than exogenous selection, and is expected to result in a very limited amount of neutral introgression. However, under a view of hybridization as a creative evolutionary force, some of the introduced genetic variation is expected to be favored by exogenous selection (at least in some habitats), and would result in levels of introgression in excess of that predicted by a neutral model.

Understanding the evolutionary significance of introgressive hybridization has become of greater interest because of the recognition that habitat alteration and population fragmentation can promote interspecific hybridization by causing a breakdown of environmental or ecological barriers to interspecific matings (Lehman *et al.* 1991; Boyd and Houpt 1994; Karl *et al.* 1995). If hybridization is largely a maladaptive process, then conservation dependent populations may be further challenged with outbreeding depression (Ellstrand and Elam 1993; Rhymer and Simberloff 1996). However, if hybridization is a creative evolutionary process, then conservation dependent populations may benefit from the introduction of novel genetic variation through hybridization, which may allow these populations to adapt to future environmental changes (Grant and Grant 1992; Rhymer and Simberloff 1996; Arnold 1997; Dowling and Secor 1997). In addition, hybridization may lead to increased effective population sizes, decreasing the potential for inbreeding depression and population extinction, and allowing for greater amounts of native genetic variation to be retained (Rhymer and Simberloff 1996; Arnold 1997).

Tests of the predictions of alternative theories regarding the role of introgressive hybridization in evolution and its impact on conservation dependent populations have been limited by the practical difficulty of identifying cases of introgression and by a lack of historical and biological details required to generate explicit null models and neutral expectations. However, the recent history of hybridization between *Bison bison* (North American bison) and *Bos taurus* (domestic cattle) provides a nearly unique opportunity to

examine the outcome of introgressive hybridization within the context of well documented historical circumstances.

At the end of the last century, North American bison experienced a dramatic demographic bottleneck in which the species was reduced from tens of millions of individuals (Seton 1929) to less than 1000 individuals in the course of less than 50 years (Hornaday 1913). During the early stages of the conservation effort, many of the populations which would eventually serve as the foundation for the approximately 300,000 North American bison in existence today (Yorks and Capels 1998), were managed in captivity, where introgressive hybridization with domestic cattle was either promoted or simply allowed to occur (Jones 1907; Boyd 1914; Goodnight 1914; Coder 1975; Dary 1989). Once public agencies took over the management of these populations, further hybridization was usually prevented (Coder 1975). However, this early history of hybridization led to the introgression of domestic cattle genes into the founding stocks of many current North American bison populations.

Introgression has been documented in eight current North American bison populations, as described in the previous two chapters. Details regarding natural history, ecology, and genetics (including a well defined genetic linkage map for domestic cattle) are available for North American bison and domestic cattle (Bishop *et al.* 1994; Berger and Cunningham 1994; Kappes *et al.* 1997; Nowak 1999). In addition, the viability and fertility of various hybrid classes, resulting from matings between the two species, has been estimated (Boyd 1908, 1914). Finally, the Custer State Park (CSP) population of North American bison has a direct history of hybridization and sufficient historical information available to generate an explicit null model of neutral introgression.

In this study, the expected frequency distribution for domestic cattle introgression under a null model of neutral introgression is generated via simulation using the known history of the CSP population. This model incorporates endogenous selection against hybrids, but is neutral with respect to exogenous selection. By determining the role that exogenous selection has played in shaping the outcome of the historically documented hybridization events in the population from which CSP was founded, the predictions of alternative theories regarding the significance of introgressive hybridization in evolution are tested. In addition, a simulation model based on the history of hybridization in the CSP population is used to evaluate the hypothesis that introgressive hybridization can result in greater retention of native genetic variation, and can decrease the probability of population extinction.

Materials and Methods

As described in the previous two chapters, the frequency of domestic cattle introgression in the CSP population has been determined for a set of 20 autosomal loci, a locus on the X chromosome, and a mitochondrial marker. In order to determine if exogenous selection has impacted the frequency of introgression at any of these loci, the expected frequency of introgression was determined using a simulation model based on the history of the CSP population (Appendix B), the fertility and viability of various hybrid classes, and information on the natural history and ecology of North American bison.

Using the general model parameters described in Appendix C, simulations were performed in cycles of reproduction, random death, population aging, calving, and artificial culling. These simulations were run for a total of 107 cycles, representing the 107 years between a detailed census in 1888, and the date of sample collection in 1995. For each of 1000 simulation replicates, the frequency of domestic cattle alleles at a set of 20 autosomal loci was determined. In addition, the maximum frequency of introgression at any one of these autosomal loci, and the frequency of domestic cattle introgression at the locus on the X chromosome was determined in each simulation replicate. The frequency of domestic cattle mtDNA haplotypes was also determined for each of the simulation replicates. Simulations in which the population went extinct, or in which there were not enough individuals to form the CSP population were discounted. The expected distributions for each of the measures described above, were generated by combining the results from each of the simulation replicates that were completed.

Sex ratios in captive North American bison populations are often highly skewed through the culling of males on the basis of various selective criteria. The potential impact of artificial selection for males with traits derived from a domestic cattle ancestry was assessed by adjusting the general model parameters described in Appendix C, so that males with a domestic cattle allele at a locus under selection were half as likely to be culled from the population. Measures of introgression were then compared with those expected under the original model, which was neutral with respect to exogenous selection.

In order to determine if hybridization with domestic cattle could have had any impact on the retention of native genetic variation or the probability of population extinction, a second series of simulations was performed. These simulations followed the parameters used in phase 1 of the previous set of simulations (Appendix C), but began

with the five original North American bison that Fredrick Dupree captured in 1883. In addition, instead of following the history of the CSP population directly, the population was simply allowed to grow until it reached 1000 individuals. In order to keep track of the number of founder alleles remaining in the population at the end of each simulation replicate, every allele in each of the five founders was uniquely identified for a set of 200 autosomal loci. At the end of 1000 simulation replicates, the number of founder alleles present in the population was averaged over all 200 loci and all completed simulation replicates.

A simulation replicate was not completed if the population went extinct. However, the number of such events was counted over the 1000 simulations performed. The frequency of extinction events and the mean percentage of founder alleles remaining in the population at the point when it reached 1000 individuals were compared between simulations in which domestic cattle were available for hybridization (as described in Appendix C), and those in which no hybridization occurred. In addition, because normal female fecundity is quite high in North American bison (0.7 calves per year), additional simulations were performed with normal female fecundity set to 0.45 calves per year, approximating the fecundity of outbred *Bison bonasus* (Slatis 1960). This was done in order to determine if hybridization would have a greater impact on populations which were not expanding as rapidly as the CSP population of North American bison.

Results

In order to test for the effects of exogenous selection, a total of 1000 simulation replicates were run using the general model parameters described in Appendix C. In three of these replicates, there were too few individuals available to simulate the foundation of the CSP population. Therefore, a total of 997 simulation replicates were used to generate expected frequency distributions for measures of domestic cattle introgression. The observed frequency of domestic cattle mtDNA haplotypes was slightly less than the mean expected frequency from simulations under a neutral model (Figure 6). However, this difference was not statistically significant ($P < 0.1$), indicating that exogenous selection did not play a significant role in the introgression of domestic cattle mtDNA haplotypes in the CSP population. Similar results were obtained for comparisons of the observed and expected frequency of autosomal loci with domestic cattle alleles (Figure 7), the total frequency of domestic cattle alleles identified across all 20 autosomal loci (Figure 8), and the maximum frequency of domestic cattle alleles at any one of the 20 autosomal loci

(Figure 9). No domestic cattle alleles were observed at the locus on the X chromosome. However, the mean expected frequency of domestic cattle alleles at this locus was not significantly ($P < 0.1$) different from zero (Figure 10). Although the observed levels of nuclear introgression were lower than the expected values for all four measures of nuclear introgression, none of these differences was statistically significant ($P < 0.1$). Therefore, there is no evidence that exogenous selection played a significant role in the introgression of domestic cattle nuclear genes in the CSP population.

The potential impact of artificial selection on expected levels of introgression was assessed by reducing the probability that a given male would be culled from the population if he possessed a domestic cattle allele at a specified autosomal locus. The mean frequency of domestic cattle alleles expected at the locus under selection (42.68%) exceeded the critical value ($P < 0.05$) for the maximum number of domestic cattle alleles expected at any single autosomal locus under a neutral model (30.77%). In fact, under the model that incorporated selection, the frequency of domestic cattle alleles expected at the selected locus was less than 30.77% in only 1% of the simulation replicates. In the simulations, the locus under selection was not one of the 20 autosomal loci used to generate expected frequency distributions for measures of introgression. While this indirect selection did increase the mean expected values for all measures of introgression (Table 10), the mean values obtained under the model with selection did not exceed the critical values ($P < 0.1$) for the distributions obtained under a neutral model. Therefore, it is unlikely that selection for domestic cattle variation would be detected as an observed excess of domestic cattle introgression unless selection was acting on one of the loci being examined, or a closely linked locus.

The results of four simulation experiments, aimed at investigating the impact of introgressive hybridization on the retention of native genetic variation and the probability of population extinction, are shown in Table 11. In both sets of simulations where hybridization with domestic cattle was permitted, the number of extinction events was reduced relative to simulations in which no hybridization occurred. When female fecundity was assumed to be 0.7 calves per year, the magnitude of this difference was small. However, when female fecundity was assumed to be 0.45 calves per year, extinction events were almost twice as likely in simulations without hybridization. However, extinctions were relatively rare in all four of the simulation experiments, occurring in less than 5% of simulation replicates. The average percentage of original North American bison alleles per locus was also determined at the end of each of the four

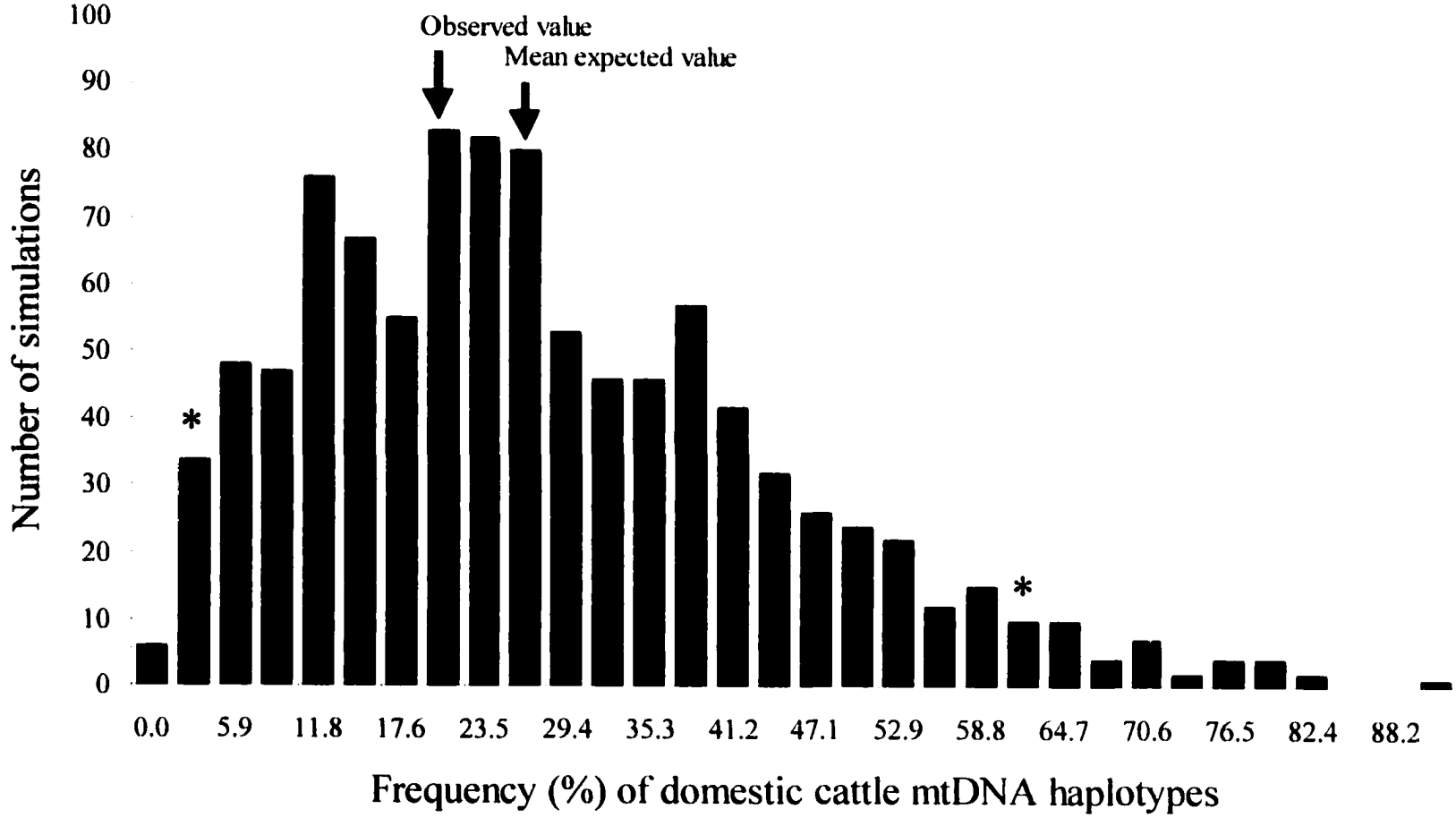

Figure 6. -Distribution of expected values for the frequency of domestic cattle mtDNA haplotypes identified in a sample of 34 North American bison from Custer State Park. Critical values ($P < 0.1$) are represented with an asterisk (*).

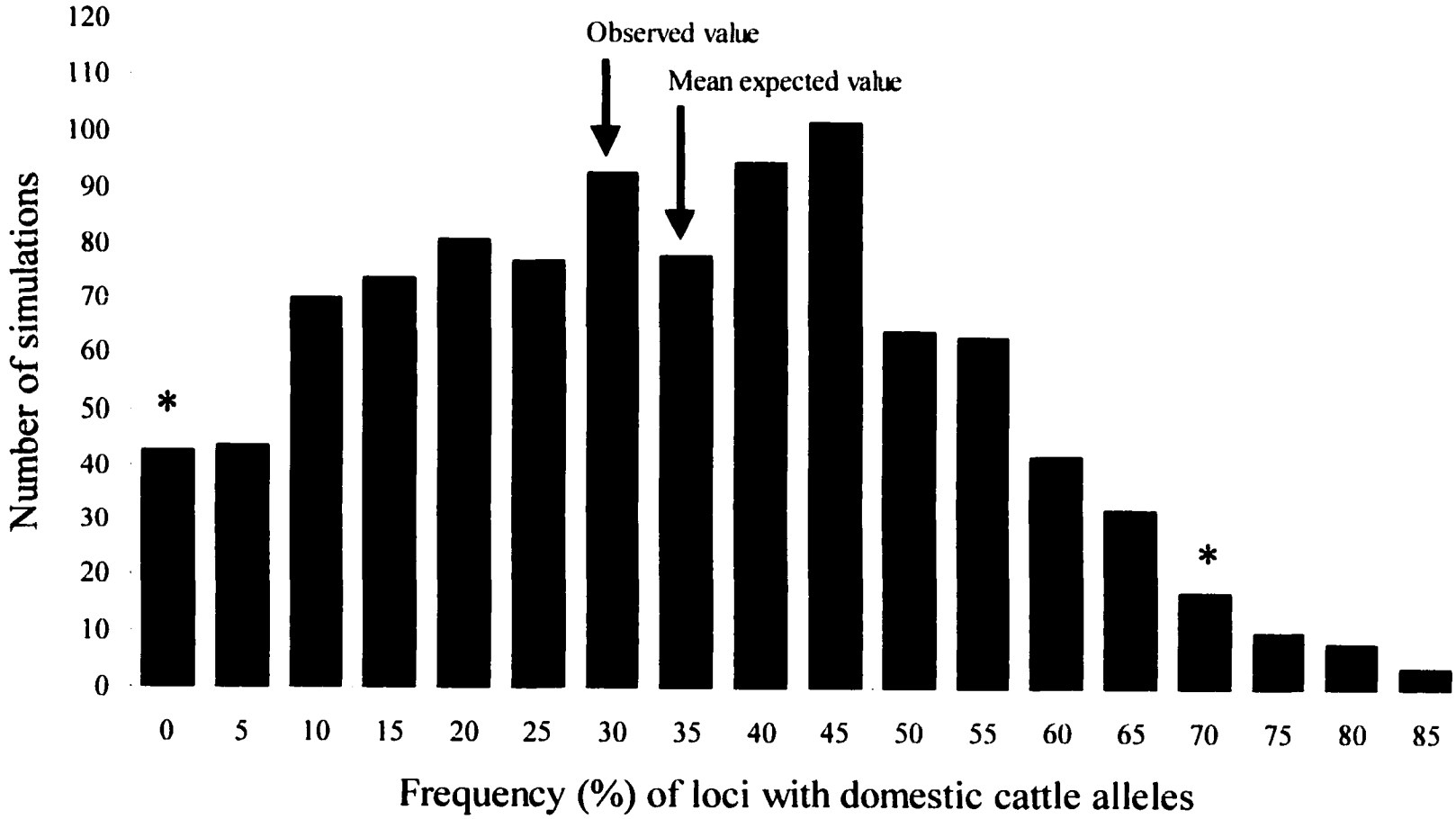

Figure 7. -Distribution of expected values for the frequency of loci with domestic cattle alleles in a sample of 20 autosomal loci. Critical values ($P < 0.1$) are represented with an asterisk (*).

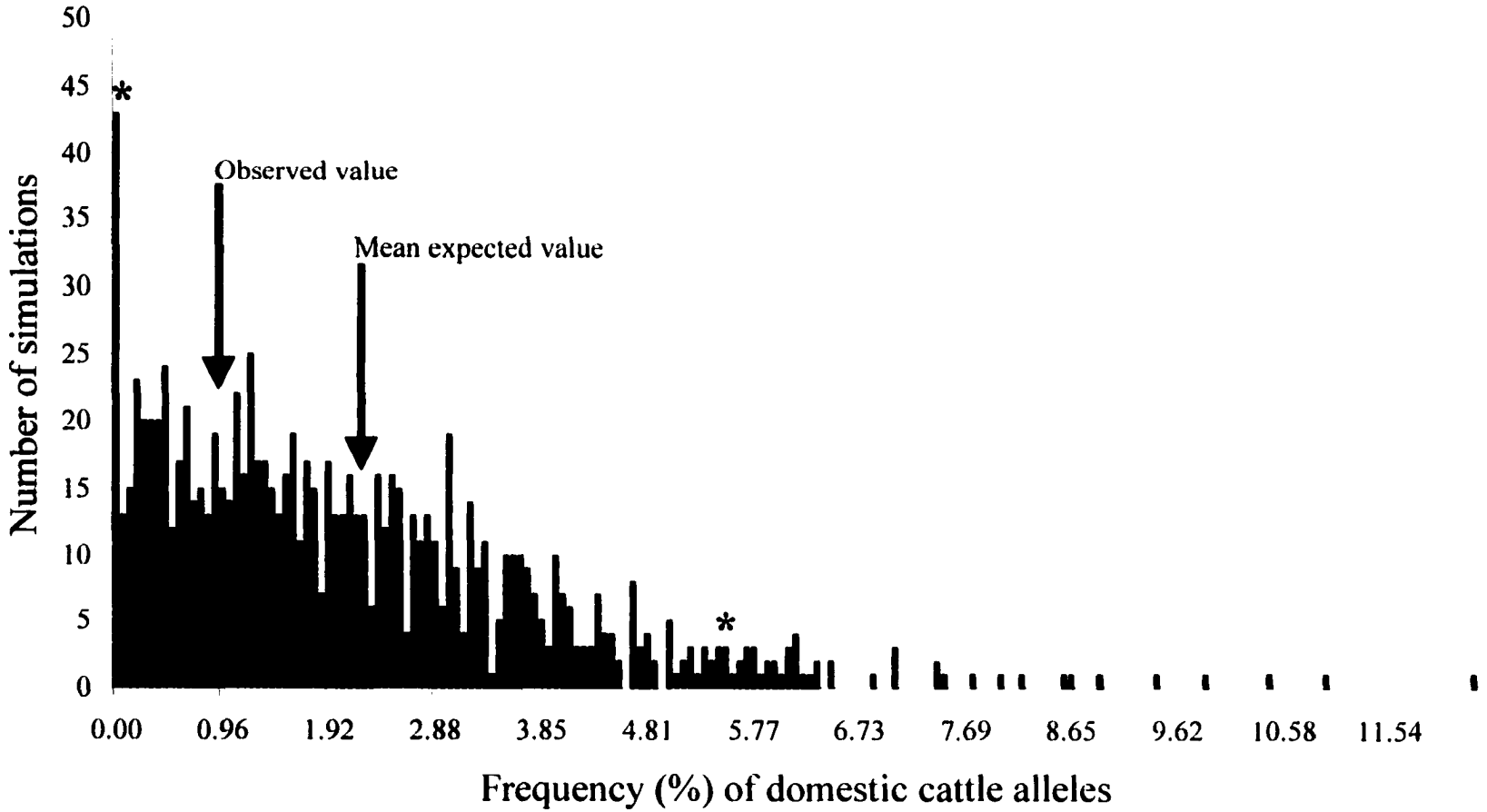

Figure 8. -Distribution of expected values for the frequency of domestic cattle alleles identified across all 20 autosomal loci examined. Critical values ($P < 0.1$) are represented with an asterisk (*).

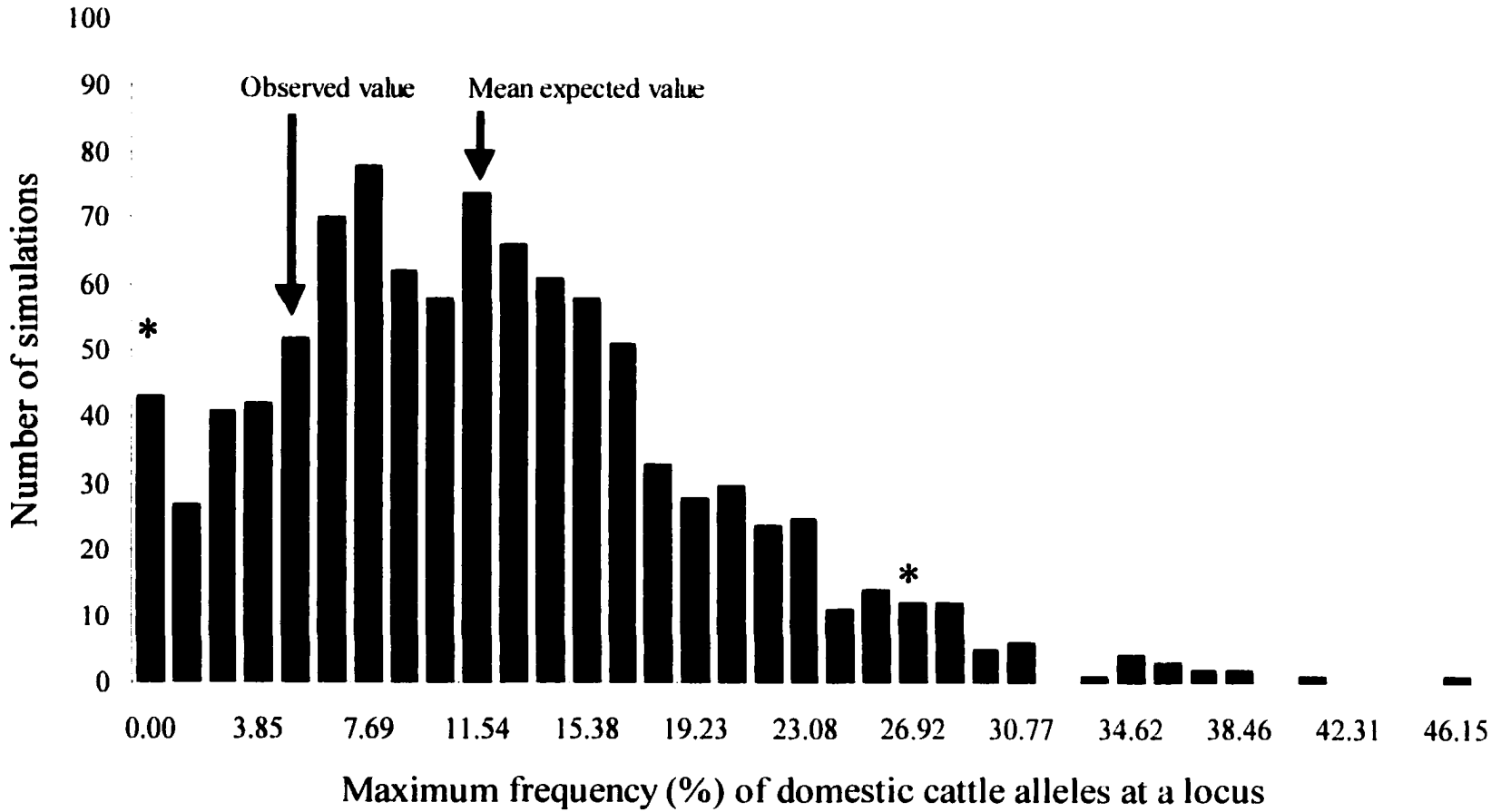

Figure 9. -Distribution of expected values for the maximum frequency of domestic cattle alleles identified at any one of the 20 autosomal loci examined. Critical values ($P < 0.1$) are represented with an asterisk (*).

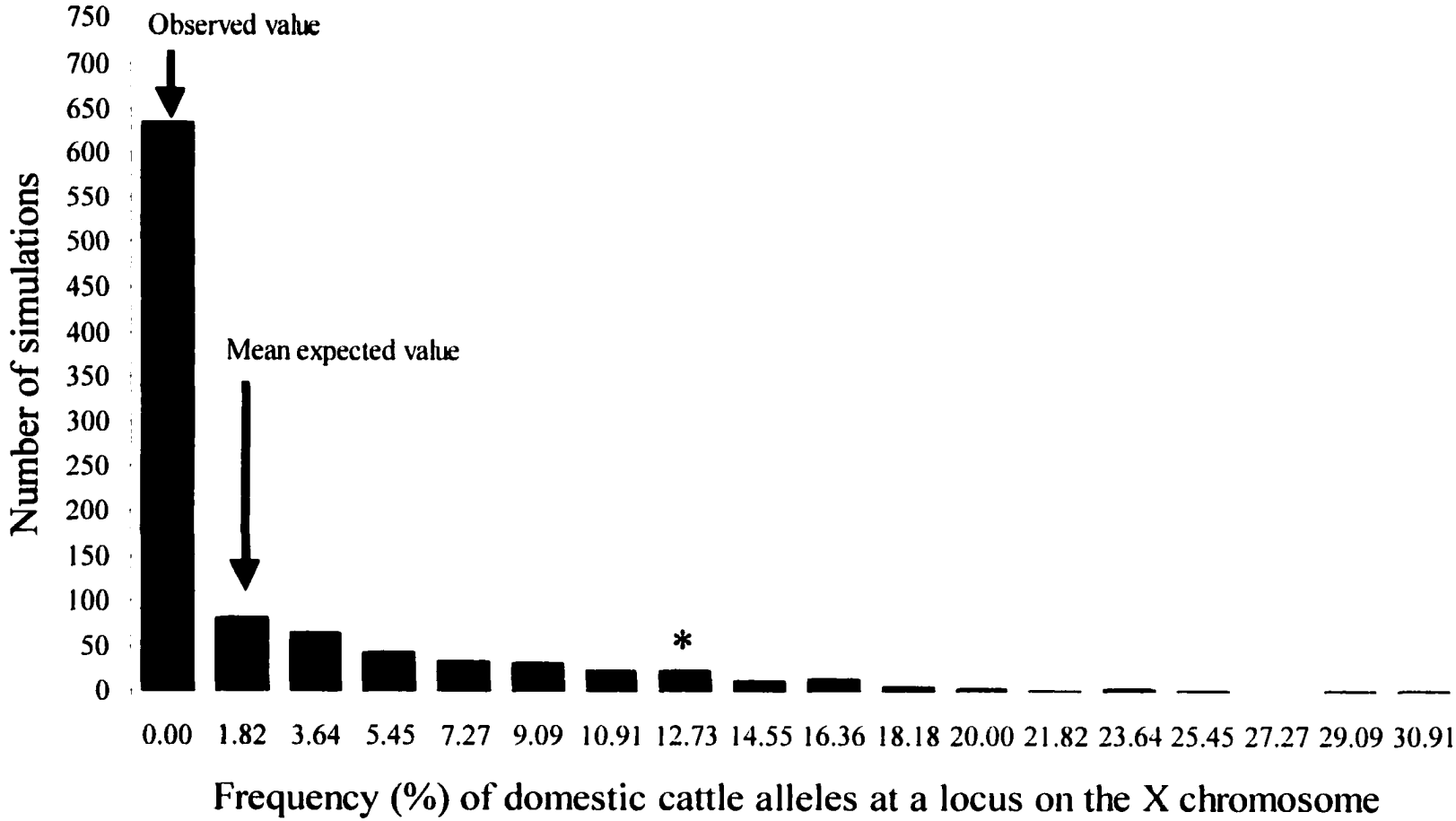

Figure 10. -Distribution of expected values for the frequency of domestic cattle alleles identified at the single X chromosome marker. The mean expected value was not significantly different from zero ($P < 0.1$). The one-tailed critical value ($P < 0.1$) is represented with an asterisk (*).

TABLE 10

Mean expected frequency (%) of introgression

Measures of Introgression	Neutral Model	Model with Selection
Frequency of DC mtDNA	27.68 ± 16.53	27.82 ± 16.44
Frequency of autosomal loci with DC alleles	33.75 ± 19.10	37.70 ± 18.95
Frequency of DC alleles across all autosomal loci	2.11 ± 1.75	2.65 ± 1.98
Maximum frequency of DC alleles at an autosomal locus	12.08 ± 7.60	14.56 ± 8.45
Frequency of DC alleles at an X-linked locus	2.78 ± 5.16	3.95 ± 6.78
Frequency of DC alleles at an autosomal locus under selection	Not applicable	42.68 ± 32.35

DC, domestic cattle.

TABLE 11

The impact of hybridization on the retention of native genetic variation and the probability of extinction

Simulation Model	Frequency (%) of Extinctions	B_N (%)
No hybridization, $F_f = 0.7$	1.6	79.6 ± 14.4
Hybridization, $F_f = 0.7$	1.4	82.2 ± 13.7
No hybridization, $F_f = 0.45$	4.6	67.2 ± 15.0
Hybridization, $F_f = 0.45$	2.4	71.4 ± 13.5

B_N, mean percent of original North American bison alleles present in the population at the end of 1000 simulation replicates (averaged over 200 loci). F_f, female fecundity, expressed as the average number of calves produced per year.

simulation experiments. In both pairs of simulations, a larger fraction of the original North American bison genetic variation was retained with hybridization (Table 11). However, the magnitude of these differences was quite small. The percentage of native North American bison alleles retained in simulations with hybridization was only 4.2% greater than without hybridization when female fecundity was assumed to be 0.45 calves per year, and was only 2.6% greater when female fecundity was assumed to be 0.7 calves per year.

Discussion

Much of the attention paid to the study of hybridization has involved examinations of hybrid zone dynamics (Endler 1977, Moore 1977, Barton and Hewitt 1985, Harrison 1993). While these studies have provided insights into processes related to speciation, they may not be the best models for examining the evolutionary significance of hybridization itself. The theoretical framework for the majority of hybrid zone studies includes the view that hybrid zones are maintained in a balance between dispersal and selection against hybrid unfitness independent of the environment, as described by Barton and Hewitt (1985). Under this view of hybrid zone dynamics, introgression is typically attributed to the neutral diffusion of characters across a "semipermeable" boundary (Key 1968). Differential introgression is used as evidence for the action of selection, but because of the spatial and temporal complexity exhibited by natural hybrid zones, it is not usually possible to develop explicit null models.

Without the ability to formulate explicit null models, it is not possible to falsify the null hypothesis of neutral introgression (Clark 1985), and multiple scenarios can be used to explain the same observations. For example, in a study of a narrow hybrid zone between two races of the Australian grasshopper *Caledia captiva*, Marchant (1988) found mtDNA haplotypes from the Moreton race 200 km further into the territory of the Torresian race than any other introgressant character examined. This pattern of cytoplasmic introgression in the absence of introgression of nuclear elements has been found in many hybridizing taxa (Ferris *et al.* 1983; Tegelstrom 1987; Whittemore and Schaal 1991; Dorado *et al.* 1992; Riesberg and Wendel 1993), and is usually attributed to founder events, hybridization asymmetries, or random fixation of mtDNA lineages due to the smaller effective population sizes of haploid mtDNA genomes. However, Marchant (1988) noted that the pattern of introgression observed in *C. captiva* could also be explained by movement of the hybrid zone in conjunction with negative selection against

nuclear genes in foreign genetic backgrounds, while Arnold (1997) argued that this pattern could be the result of positive selection for Moreton mtDNA haplotypes in a Torresian genetic background.

An examination of the outcomes of hybridization between domestic cattle and North American bison benefits from the ability to generate explicit neutral expectations, making it possible to test for the effects of exogenous selection on levels of introgression. The fact that hybridization events were coincident with a major population bottleneck (the forerunner to the CSP population was started with only five North American bison) and with the partial domestication of North American bison would seem to provide the ideal circumstances under which introgressive hybridization could serve as a creative evolutionary force. However, the hypothesis that hybridization with domestic cattle provided North American bison with critically needed genetic variation, that was either lost during the bottleneck, or which was necessary to adapt to domestication, is not supported by the analyses presented here. Observed levels of introgression were consistent with neutral expectations, indicating that domestic cattle variation was not selectively retained in the CSP population of North American bison. However, it should be noted that the analyses presented here also indicate that the impact of selection for domestic cattle variation at a locus not closely linked to the loci examined would likely have gone undetected.

While positive selection for domestic cattle variation at a locus not tightly linked to those examined is a possibility, the greatly reduced fitness of early generation hybrids clearly indicates that some regions of North American bison and domestic cattle genomes are incompatible. Introgression would have been strongly selected against in these regions. The null model described in Appendix C, incorporates selection against hybrids, but assumes that the chromosomal regions examined were not closely linked to genes causing hybrid inviability or infertility. The fact that observed levels of introgression were consistent with the expectations of this model, indicates that the strong selection against incompatible genes in hybrid individuals was not broadly distributed across the genome. Obviously, many more genomic segments need to be examined, but this result could indicate that there are only a small number of genes or genomic regions responsible for the species barrier between North American bison and domestic cattle.

In addition to the fact that traditional analyses of hybrid zones may not provide the best framework for examining the evolutionary significance of hybridization, they may also fail to reflect the dynamics of hybridization and introgression in small fragmented

populations that have come into contact with a more numerous and reproductively compatible taxon. However, circumstances surrounding historical hybridization events between North American bison and domestic cattle are typical of those experienced by many conservation dependent taxa. For example, human activities have resulted in population decline and fragmentation of all wild cattle species (National Research Council 1983). Domestic cattle are interfertile with wild cattle and have been introduced into areas inhabited by all wild cattle species (Nowak 1999). Most populations of *Bos javanicus* (banteng) are actually hybrid swarms, formed by centuries of hybridization with domestic cattle (National Research Council 1983), and hybrids have also been identified among the last wild populations of *Bos grunniens* (yak) (Schaller and Wulin 1996).

When a conservation dependent taxon experiences gene flow from a more numerous taxon, genetic assimilation is a major concern (Ellstrand and Elam 1993). Genetic assimilation can occur through asymmetric gene flow from the more common taxon (Arnold 1997), but the strong endogenous selection against hybrids formed through crosses between domestic cattle and North American bison probably prevented this from occurring in populations such as the one that was used to found the CSP population. In addition, behavioral constraints appear to have limited hybridization to crosses involving male North American bison and female domestic cattle (Boyd 1908, 1914; Goodnight 1914; Coder 1975; Dary 1989). This sexual asymmetry in the direction of hybridization may also have helped North American bison avoid genetic assimilation, by slowing the rate at which hybrids were formed relative to matings between pure North American bison. Therefore, an understanding of the relative fitness of hybrids and the recognition of mating asymmetries may aid in the development of plans to deal with hybridization in the management of other conservation dependent species.

A second concern for conservation dependent populations exposed to reproductively compatible taxa is a reduction in population fitness and the waste of reproductive potential through outbreeding depression (Ellstrand and Elam 1993; Rhymer and Simberloff 1996). There is no indication that the forerunner to the CSP population suffered from outbreeding depression despite years of hybridization with domestic cattle that produced relatively unfit hybrid progeny. In fact, in simulations based on the history of hybridization in this population, fewer extinctions occurred when hybridization with domestic cattle was incorporated than when no hybridization occurred. In addition, a greater amount of native genetic variation was retained when hybridization was incorporated than when no hybridization occurred. These results are consistent with

predictions based on a view of introgressive hybridization as a creative evolutionary force. However, in all of the simulations performed, the population was growing too rapidly for extinction or the loss of native variation through genetic drift to be of major concern, and the differences between simulations with hybridization and those without hybridization were small. The magnitude of these differences did increase in simulations with reduced female fecundity, indicating that hybridization could play a more important role in reducing the chances of population extinction and the loss of native genetic variation in populations with low rates of intrinsic growth, such as those that have been severely effected by inbreeding depression. However, these populations could also be more susceptible to genetic assimilation.

The ability to generate an explicit null model of neutral introgression allowed for a test of the predictions of alternative theories regarding the significance of hybridization in evolution. Predictions about the role of exogenous selection in shaping the outcome of hybridization events, based on the view of hybridization as a creative evolutionary force, were not supported. However, there is some indication that hybrids can act to decrease the probability of extinction and to preserve native genetic variation. Ultimately, there is no indication that hybridization played any significant role in the recovery of North American bison from a dramatic population bottleneck, but there is also no indication that it was maladaptive in any way. However, the fact that a limited pulse of hybridization that occurred over 100 years ago resulted in introgression that was detected at 30% of the autosomal loci examined, indicates that introgressive hybridization could have more than a transient effect on the evolution of hybridizing species, even when there is strong endogenous selection against hybrid individuals.

SUMMARY AND CONCLUSIONS

Synopsis

One of the greatest challenges in testing the predictions of alternative theories regarding the role of introgressive hybridization in evolution involves identifying instances where introgression has occurred and distinguishing introgression from symplesiomorphy and homoplasy. In order to determine if the historically documented hybridization events that took place between North American bison and domestic cattle resulted in introgression, diagnostic molecular markers from both the mitochondrial and nuclear genomes were identified. In the mitochondrial genome, a set of domestic cattle specific primers was identified from control region sequences of a number of bovine species. These primers were used to identify a total of four unique domestic cattle mtDNA haplotypes in seven of the 16 North American bison populations examined. A comparison of haplotype data with historical records was used to determine that the Custer State Park, Caprock Canyon State Park, and the Finney / Maxwell Game Refuge populations represented three independent centers of mtDNA introgression. Phylogenetic patterns produced from haplotype data, along with an examination of levels of sequence divergence, allowed mtDNA introgression to be distinguished from symplesiomorphy and homoplasy.

The introgression of domestic cattle chromosomal segments was identified in seven different regions of the North American bison genome, and in five different North American bison populations, including a population where no mtDNA introgression had been identified. Data from sets of closely linked microsatellite markers was used to distinguish introgression from allele size homoplasy and symplesiomorphy. This approach, makes it possible to use the vast number of microsatellite markers in eukaryotic genomes to identify introgression, determine the size of introgressant segments, and to identify the location of these segments throughout the genome. The large number of diagnostic markers employed in this study made it possible to identify North American bison populations with appreciable levels of introgression, and also allowed for levels of introgression to be quantified in different parts of the North American bison genome.

A second challenge in determining if introgressive hybridization can play a significant role in evolution, involves the generation of an explicit null hypothesis of neutral introgression. This is not possible in most naturally occurring hybrid zones, because the necessary historical and biological information is generally lacking. Using a

variety of historical and biological information, the history of hybridization and the population dynamics of the Custer State Park population were simulated, making it possible to generate explicit neutral expectations regarding the levels of introgression in this population. The observed levels of introgression were consistent with neutral expectations, indicating that domestic cattle variation had not been selectively maintained in this population. However, these analyses were restricted to an examination of introgression in the Custer State Park population. The pattern of introgression in the Fort Niobrara population indicated that domestic cattle variation at a gene known to effect growth and conformation characteristics in domestic cattle may have been selectively maintained. This pattern may also be the result of a sampling event related to a secondary introduction of domestic cattle genes into the Fort Niobrara population via a recent transfer of North American bison with a hybrid ancestry from another population. Additional analysis of introgression in this, and other North American bison populations, would be beneficial if additional information on the history of hybridization in these populations could be obtained.

Interest in the evolutionary significance of hybridization and introgression has grown among conservation biologists because of the recognition that hybridization is often a secondary consequence of habitat modification and population fragmentation, and may pose an additional threat to conservation dependent populations. The history of hybridization in North American bison populations, provides an opportunity to examine the dynamics of hybridization in conservation dependent populations. The results of simulation analyses based on the history of hybridization in the Custer State Park population, indicated that hybridization could act to slightly reduce the probability of population extinction and the loss of native genetic variation under some circumstances, even when hybrid progeny were relatively unfit. The outcome of hybridization in the Custer State Park population also indicates that a relatively strong species barrier and hybridization asymmetries can prevent genetic assimilation, even when extremely small populations come into contact with a more numerous and reproductively compatible taxon.

Implications for the Future Management of North American Bison

The results of this study provide no evidence that hybridization with domestic cattle had any impact on the recovery of North American bison from a major demographic decline during the nineteenth century. However, domestic cattle introgression was identified in eight of the 16 populations examined. How these results, should influence

the future management of North American bison is largely dependent on the rationale behind the management of individual North American bison populations. Clearly, there is no indication that the presence of domestic cattle genes poses any threat to the fitness or future productivity of the North American bison populations examined. Therefore, if the goal of a management plan is to simply maintain a viable population of North American bison, the introgression documented here should be of limited concern. However, the introgression of chromosomal segments spanning several centimorgans was identified in five of the North American bison populations examined, and in the Custer State Park population, domestic cattle chromosomal segments as large as eight centimorgans were identified at 30% of the autosomal loci examined. While there is no indication that variation within these chromosomal segments has been selectively maintained or has had a negative impact on fitness, this does not mean that introgression has had no phenotypic effect on North American bison. Therefore, if the preservation of native North American bison genetic diversity and unique North American bison phenotypes is a goal of population management, the impact of introgression should be considered, and care should be taken so that management practices do not result in the replacement of native North American bison variation with that of domestic cattle.

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APPENDIX A

AMPLIFICATION PROTOCOLS

1. Amplifications were performed in 5µl reactions with 0.8µM BYM1 primers, 2.5mM $MgCl_2$, 0.5mM dNTP's, and 0.375 U of Taq polymerase (Promega). Polymerase chain reactions (PCR) consisted of an initial denaturation at 94° (4 min), then 35 cycles of 94° (20 sec), 66° (20 sec), and 72° (20 sec), followed by a final extension at 72° (20 min).

2. Amplifications were performed in 10µl reactions with 0.6µM BMS1172, 0.6µM RM372, 0.4µM BMS410, 0.4µM BMS2639, 0.6µM BMS1117, and 0.7µM BMS1862 primers, 3.0mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO, and 0.5 U Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (3 min), then four cycles of 94° (30 sec), 58° (15 sec), and 72° (5 sec), then 31 cycles of 94° (15 sec), 56° (15 sec), and 72° (2 sec), followed by a final extension at 72° (20 min).

3. Amplifications were performed in 10µl reactions with 0.3µM BMS527, 0.2µM BM2113, 0.25µM BM17132, and 0.2µM BMS510 primers, 3.0mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO, and 0.5 U Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (3 min), then four cycles of 94° (30 sec), 58° (15 sec), and 72° (5 sec), then 31 cycles of 94° (15 sec), 56° (15 sec), and 72° (2 sec), followed by a final extension at 72° (20 min).

4. Amplifications were performed in 10µl reactions with 0.14µM BM4440, 0.8µM BM720, 0.2µM BM1706, 0.2µM BM1225, and 0.14µM BM1905 primers, 2.5mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO, and 0.5 U Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (3 min), then four cycles of 94° (30 sec), 58° (15 sec), and 72° (5 sec), then 31 cycles of 94° (15 sec), 56° (15 sec), and 72° (2 sec), followed by a final extension at 72° (20 min).

5. Amplifications were performed in 10µl reactions with 0.2µM BMS460 primers, 2.5mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO and 0.5 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (4 min), then three cycles of 94° (30 sec), 55° (15 sec), and 72° (5 sec), then 35 cycles of 92° (15 sec), 54° (15 sec), and 72° (2 sec), followed by a final extension at 72° (20 min).

6. Amplifications were performed in 10µl reactions with 0.14µM BMS1116, 0.6µM BMS555, 0.6µM BMS1128, 0.14µM CSSM36, and 0.4µM BMS903 primers, 3.75mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO, and 1.0 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (4 min), then five cycles of 94° (30 sec), 58° (15 sec), and 72° (5 sec), then 35 cycles of 94° (15 sec), 56° (15 sec), and 72° (2 sec), followed by a final extension at 72° (20 min).

7. Amplifications were performed in 10µl reactions with 0.2µM TGLA57, 0.9µM CSSM46, 0.2µM BMS585, 0.4µM BM203, and 0.3µM BM4602 primers, 3.0mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO, 1%BSA, and 1.0 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (4 min), then six cycles of 94° (30 sec), 58° (15 sec) - 0.5° per cycle, and 72° (5 sec), then 27 cycles of 94° (30 sec), 54° (15 sec), and 72° (2 sec) + 1 sec per cycle, followed by a final extension at 72° (20 min).

8. Amplifications were performed in 10µl reactions with 0.6µM RM500 and 0.6µM TGLA227 primers, 2.5mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO, and 0.5 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (3 min), then three cycles of 94° (30 sec), 54° (20 sec), and 72° (5 sec), then 37 cycles of 94° (15 sec), 53° (10 sec), and 72° (3 sec), followed by a final extension at 72° (20 min).

9. Amplifications were performed in 10µl reactions with 0.6µM BMS947, 0.4µM BM4513, and 0.4µM AGLA29 primers, 2.5mM $MgCl_2$, 0.5mM dNTP's, and 1.0 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (4 min), then six cycles of 94° (30 sec), 58° (15 sec) - 0.5° per cycle, and 72° (5 sec), then 27 cycles of 94° (30 sec), 54° (15 sec), and 72° (2 sec) + 1 sec per cycle, followed by a final extension at 72° (20 min).

10. Amplifications were performed in 5µl reactions with 0.7µM BM2830, 0.3µM BM4311, 0.6µM BMS2840 primers, and 0.48µM BMS2533 primers, 3.0mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO, 1% BSA, and 0.25 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (2 min), then four cycles of 94° (30 sec), 58° (15 sec) - 0.5° per cycle, and 72° (5 sec), then 35 cycles of 92° (15 sec), 54° (15 sec), and 72° (2 sec), followed by a final extension at 72° (20 min).

11. Amplifications were performed in 5µl reactions with 0.3µM HUJ246, 0.6µM BMS2809, 0.6µM BMS1716, 0.3µM BM6507, 0.4µM BMS2142, and 0.3µM BMS2270 primers, 3.25mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO, 1% BSA, and 0.25 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (2 min), then six cycles of 94° (30 sec), 58° (15 sec) - 0.5° per cycle, and 72° (5 sec), then 29 cycles of 94° (15 sec), 54° (15 sec), and 72° (5 sec), followed by a final extension at 72° (20 min).

12. Amplifications were performed in 10µl reactions with 0.5µM BM1824, 0.4µM BMS360, 0.3µM BMS2258, 0.5µM BMS1979, and 0.2µM BMS2269 primers, 3.0mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO, 1% BSA, and 0.5 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (2 min), then four cycles of 94° (30 sec), 58° (15 sec) - 0.5° per cycle, and 72° (5 sec), then 26 cycles of 94° (15 sec), 54° (15 sec), and 72° (5 sec), followed by a final extension at 72° (20 min).

13. Amplifications were performed in 10µl reactions with 0.3µM BMS827, 0.6µM BM2934, and 0.3µM BM3517 primers, 3.0mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO, 1% BSA, and 0.5 U of Taq polymerase (Promega). PCR consisted of an initial denaturation

at 94° (2 min), then four cycles of 94° (30 sec), 58° (15 sec) - 0.5° per cycle, and 72° (5 sec), then 26 cycles of 94° (15 sec), 54° (15 sec), and 72° (5 sec), followed by a final extension at 72° (20 min).

14. Amplifications were performed in 5µl reactions with 1.12µM BMS4031, 0.52µM IL4, 0.12µM BMS1967, 0.32µM BMS2047, and 0.52µM BMS820 primers, 2.5mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO, 1% BSA, and 0.25 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (2 min), then four cycles of 94° (30 sec), 59° (15 sec) - 0.5° per cycle, and 72° (5 sec), then 29 cycles of 92° (15 sec), 54° (15 sec), and 72° (5 sec), followed by a final extension at 72° (20 min).

15. Amplifications were performed in 10µl reactions with 0.8µM BMC701, 0.6µM BMS419, and 0.6µM BMS130 primers, 3.0mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO, 1% BSA, and 0.5 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (2 min), then four cycles of 94° (30 sec), 58° (15 sec) - 0.5° per cycle, and 72° (5 sec), then 26 cycles of 94° (15 sec), 54° (15 sec), and 72° (5 sec), followed by a final extension at 72° (20 min).

16. Amplifications were performed in 5µl reactions with 0.3µM BMS4040 and 0.6µM BMS2349 primers, 3.0mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO, and 0.25 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (3 min), then six cycles of 94° (30 sec), 59° (15 sec) - 0.5° per cycle, and 72° (5 sec), then 25 cycles of 94° (15 sec), 56° (15 sec), and 72° (5 sec), followed by a final extension at 72° (20 min).

17. Amplifications were performed in 5µl reactions with 0.4µM IFNAR15-2, 0.7µM CSSM22, 0.4µM BM757, 0.6µM BM121, 0.4µM BMS1743, 0.4µM BMS466, 0.3µM BMS1675, and 0.6µM OCAM primers, 3.0mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO, and 0.375 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (3 min); then six cycles of 94° (30 sec), 59° (15 sec) - 0.5° per cycle, and 72° (5 sec), then 25 cycles of 94° (15 sec), 56° (15 sec), and 72° (5 sec), followed by a final extension at 72° (20 min).

18. Amplifications were performed in 5µl reactions with 0.4µM BMS574, 0.6µM BMS610, 0.4µM ILSTS065, 0.2µM BMS528, 0.88µM BM4028, 1.0µM INRA194, and 0.84µM CSSM43 primers, 2.5mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO, and 0.375 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (2 min), then six cycles of 94° (30 sec), 58° (15 sec) - 0.5° per cycle, and 72° (5 sec), then 25 cycles of 94° (15 sec), 54° (15 sec), and 72° (5 sec), followed by a final extension at 72° (20 min).

19. Amplifications were performed in 5µl reactions with 0.28µM BMC4214, 0.3µM BMS1095, 0.24µM SPS113, 0.28µM ILSTS008, and 0.6µM BMS2055 primers, 2.5mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO, and 0.375 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (2 min), then six cycles of 94° (30 sec), 58°

(15 sec) - 0.5° per cycle, and 72° (5 sec), then 25 cycles of 94° (15 sec), 54° (15 sec), and 72° (5 sec), followed by a final extension at 72° (20 min).

20. Amplifications were performed in 5µl reactions with 0.88µM BMS1004 and 0.8µM BMS1857 primers, 3.0mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO, 1% BSA, and 0.25 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (2 min), then four cycles of 94° (30 sec), 58° (15 sec) - 0.5° per cycle, and 74° (5 sec), then 31 cycles of 92° (15 sec), 54° (15 sec), and 74° (5 sec), followed by a final extension at 72° (20 min).

21. Amplifications were performed in 5µl reactions with 0.6µM BM7145, 0.2µM BMS803, 0.6µM BMS2, 1.2µM CSN3, 0.52µM BMS2295, and 0.28µM BM4107 primers, 2.5mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO, and 0.375 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (2 min), then six cycles of 94° (30 sec), 58° (15 sec) - 0.5° per cycle, and 72° (5 sec), then 25 cycles of 94° (15 sec), 54° (15 sec), and 72° (5 sec), followed by a final extension at 72° (20 min).

22. Amplifications were performed in 10µl reactions with 0.6µM CSSM42 primers, 2.5mM $MgCl_2$, 0.5mM dNTP's, and 1.0 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (4 min), then six cycles of 94° (30 sec), 58° (15 sec) - 0.5° per cycle, and 72° (5 sec), then 27 cycles of 94° (30 sec), 54° (15 sec), and 72° (2 sec) + 1 sec per cycle, followed by a final extension at 72° (20 min).

23. Amplifications were performed in 5µl reactions with 0.8µM HEL11 and 0.28µM BL23 primers, 3.0mM $MgCl_2$, 0.5mM dNTP's, and 0.375 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (3 min), then five cycles of 94° (30 sec), 56° (15 sec), and 72° (5 sec), then 30 cycles of 94° (20 sec), 52° (15 sec), and 72° (5 sec), followed by a final extension at 72° (20 min).

24. Amplifications were performed in 5µl reactions with 0.32µM BMS1074, 0.4µM MCM74, and 0.32µM BMS911 primers, 2.5mM $MgCl_2$, 0.5mM dNTP's, and 0.25 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (3 min), then six cycles of 94° (30 sec), 58° (15 sec), and 72° (5 sec), then 25 cycles of 94° (15 sec), 56° (15 sec), and 72° (5 sec), followed by a final extension at 72° (20 min).

25. Amplifications were performed in 5µl reactions with 0.4µM BMS4023, 0.6µM NRAMP1, and 0.28µM TCRB primers, 3.0mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO, and 0.25 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (3 min), then six cycles of 94° (30 sec), 59° (15 sec) - 0.5° per cycle, and 72° (5 sec), then 25 cycles of 94° (15 sec), 56° (15 sec), and 72° (5 sec), followed by a final extension at 72° (20 min).

26. Amplifications were performed in 5µl reactions with 0.4µM BM9146 primers, 3.0mM $MgCl_2$, 0.5mM dNTP's, and 0.25 U of Taq polymerase (Promega). PCR

consisted of an initial denaturation at 94° (3 min), then six cycles of 94° (30 sec), 58° (20 sec), and 72° (5 sec), then 25 cycles of 94° (20 sec), 56° (15 sec), and 72° (5 sec), followed by a final extension at 72° (20 min).

27. Amplifications were performed in 5µl reactions with 0.8µM BM9146 primers, 3.0mM $MgCl_2$, 0.5mM dNTP's, and 0.375 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (3 min), then five cycles of 94° (30 sec), 58° (20 sec), and 72° (5 sec), then 30 cycles of 94° (20 sec), 54° (15 sec), and 72° (5 sec), followed by a final extension at 72° (20 min).

28. Amplifications were performed in 10µl reactions with 0.4µM PIT1 7B7 primers, 2.5mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO, and 1.0 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (2 min), then five cycles of 94° (20 sec), 51° (20 sec), and 72° (30 sec), then 35 cycles of 94° (20 sec), 49° (20 sec), and 72° (30 sec), followed by a final extension at 72° (14 min).

29. Amplifications were performed in 5µl reactions with 0.6µM BM4307 primers, 3.0mM $MgCl_2$, 0.5mM dNTP's, and 0.375 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (4 min), then three cycles of 94° (30 sec), 55° (20 sec), and 72° (5 sec), then 35 cycles of 92° (15 sec), 54° (15 sec), and 72° (2 sec), followed by a final extension at 72° (20 min).

30. Amplifications were performed in 5µl reactions with 0.8µM AGLA17, 0.52µM INRA119, and 0.52µM BM1314 primers, 3.0mM $MgCl_2$, 0.5mM dNTP's, and 0.375 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (3 min), then five cycles of 94° (30 sec), 58° (15 sec), and 72° (5 sec), then 30 cycles of 94° (20 sec), 54° (15 sec), and 72° (5 sec), followed by a final extension at 72° (20 min).

31. Amplifications were performed in 10µl reactions with 1.0µM AGLA293 primers, 2.5mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO, and 0.5 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (3 min), then three cycles of 94° (30 sec), 54° (20 sec), and 72° (5 sec), then 37 cycles of 94° (15 sec), 53° (10 sec), and 72° (3 sec), followed by a final extension at 72° (20 min).

32. Amplifications were performed in 10µl reactions with 0.6µM BM6438 and 0.3µM TGLA49 primers, 2.5mM $MgCl_2$, 0.5mM dNTP's, and 1.0 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (4 min), then six cycles of 94° (30 sec), 58° (15 sec) - 0.5° per cycle, and 72° (5 sec), then 27 cycles of 94° (30 sec), 54° (15 sec), and 72° (2 sec) + 1 sec per cycle, followed by a final extension at 72° (20 min).

33. Amplifications were performed in 5µl reactions with 0.8µM BMS4008 primers, 3.25mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO, and 0.25 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (2 min), then six cycles of 94° (30 sec),

58° (15 sec) - 0.5° per cycle, and 72° (5 sec), then 29 cycles of 94° (15 sec), 54° (15 sec), and 72° (2 sec), followed by a final extension at 72° (20 min).

34. Amplifications were performed in 5µl reactions with 0.8µM RM185 primers, 2.5mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO, and 0.375 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (3 min), then six cycles of 94° (30 sec), 59° (15 sec) - 0.5° per cycle, and 72° (5 sec), then 25 cycles of 94° (15 sec), 56° (15 sec), and 72° (5 sec), followed by a final extension at 72° (20 min).

35. Amplifications were performed in 10µl reactions with 0.4µM BMS1315 primers, 2.5mM $MgCl_2$, 0.5mM dNTP's, and 1.0 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (4 min), then six cycles of 94° (30 sec), 58° (15 sec) - 0.5° per cycle, and 72° (5 sec), then 27 cycles of 94° (30 sec), 54° (15 sec), and 72° (2 sec) + 1 sec per cycle, followed by a final extension at 72° (20 min).

36. Amplifications were performed in 5µl reactions with 0.32µM BM6506 and 0.4µM BM7233 primers, 2.5mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO, and 0.25 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (3 min), then six cycles of 94° (30 sec), 58° (15 sec), and 72° (5 sec), then 25 cycles of 94° (15 sec), 56° (15 sec), and 72° (5 sec), followed by a final extension at 72° (20 min).

37. Amplifications were performed in 5µl reactions with 0.2µM BM6444 and 0.6µM PRL2 primers, 3.0mM $MgCl_2$, 0.5mM dNTP's, 5% DMSO, and 0.25 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (3 min), then six cycles of 94° (30 sec), 59° (15 sec) - 0.5° per cycle, and 72° (5 sec), then 25 cycles of 94° (15 sec), 56° (15 sec), and 72° (5 sec), followed by a final extension at 72° (20 min).

38. Amplifications were performed in 5µl reactions with 0.8µM INRA117 primers, 3.0mM $MgCl_2$, 0.5mM dNTP's, and 0.25 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (3 min), then six cycles of 94° (30 sec), 58° (20 sec), and 72° (5 sec), then 25 cycles of 94° (20 sec), 56° (15 sec), and 72° (5 sec), followed by a final extension at 72° (20 min).

39. Amplifications were performed in 5µl reactions with 0.6µM BMS4017 and 0.4µM BMS4019 primers, 3.0mM $MgCl_2$, 0.5mM dNTP's, and 0.375 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (3 min), then six cycles of 94° (30 sec), 58° (20 sec), and 72° (5 sec), then 25 cycles of 94° (20 sec), 56° (15 sec), and 72° (5 sec), followed by a final extension at 72° (20 min).

40. Amplifications were performed in 5µl reactions with 0.4µM ILSTS065 and 0.4µM RM103 primers, 2.5mM $MgCl_2$, 0.5mM dNTP's, and 0.25 U of Taq polymerase (Promega). PCR consisted of an initial denaturation at 94° (3 min), then six cycles of 94° (30 sec), 58° (20 sec), and 72° (5 sec), then 25 cycles of 94° (20 sec), 56° (15 sec), and 72° (5 sec), followed by a final extension at 72° (20 min).

APPENDIX B

HISTORY OF THE CUSTER STATE PARK POPULATION

1883- Fredrick Dupree captured five calves, and raised them in captivity along with domestic cattle (Coder 1975).

1888- The population consisted of five female North American bison, four male North American bison, and seven hybrids (Coder 1975).

1898- Hybridization with domestic cattle was discontinued (Coder 1975).

1901- James Philip purchased the Dupree population. Animals with an obvious hybrid ancestry were removed from the population, and further hybridization with domestic cattle was not permitted (Coder 1975).

1914- The Custer State Park population was founded with 18 mature females, six mature bulls, and 12 calves from the Philip population (Coder 1975).

1939- Twenty-four North American bison were introduced into Custer State Park from the Wind Cave National Park (B. Muenchau, personal communication).

1940- Eleven North American bison were introduced into Custer State Park from the Wind Cave National Park (B. Muenchau, personal communication).

1953- Eighty North American bison were introduced into Custer State Park from the Wind Cave National Park (B. Muenchau, personal communication).

1954- One hundred and sixty-six North American bison were introduced into Custer State Park from the Wind Cave National Park (B. Muenchau, personal communication).

1955- One hundred and forty North American bison were introduced into Custer State Park from the Wind Cave National Park (B. Muenchau, personal communication).

1956- Eighty-five North American bison were introduced into Custer State Park from the Wind Cave National Park (B. Muenchau, personal communication).

1958- Forty-three North American bison were introduced into Custer State Park from the Wind Cave National Park (B. Muenchau, personal communication).

1959- Forty-three North American bison were introduced into Custer State Park from the Wind Cave National Park (B. Muenchau, personal communication).

1967- There were approximately 3,000 North American bison in the Custer State Park population. This represents the largest documented size of the Custer State Park population (R. Walker, personal communication).

APPENDIX C

GENERAL SIMULATION PARAMETERS

Historical Context

The simulation begins with the Dupree population as it was described in 1888, and follows the history of this population as described in Appendix B. The ages of the animals recorded in the census of 1888 were estimated using data on the reproductive potential of North American bison, as described below. All of the hybrid individuals in this original population are assumed to be F1 progeny, as insufficient time had elapsed to allow for a backcross generation to have been produced. In addition, due to the extremely low viability of male F1 hybrids, all of the hybrids in this original population are assumed to be female. Throughout the simulation, male domestic cattle are assumed to not participate in hybridization, as historical reports indicate a behavioral constraint that limited the direction of hybrid crosses (Boyd 1908, 1914; Goodnight 1914; Coder 1975; Dary 1989). The number of female domestic cattle available for hybridization was estimated from the number of hybrids produced between 1885 (when the captured North American bison would have reached sexual maturity) and 1888 (the last detailed census), and was held constant until the domestic cattle were removed from the population in 1898. In the simulations, the composition of hybrids was determined by counting the frequency of North American bison alleles present in a set of 200 loci. After 1901, individuals with North American bison alleles at less than 76% frequency across these 200 loci, were culled from the population.

North American bison are a highly polygynous species (Berger and Cunningham 1994), and the number of breeding age males is sharply reduced relative to the number of females in most populations, by culling old and immature bulls. Culling in the Dupree population is assumed to be conservative, due to the fact that this population was rebounding from a major bottleneck and was relatively small throughout the period of ownership by Dupree. Therefore, culling during this period was limited to 16 year old males, with additional culling as necessary to limit the number of mature bulls to 20. This period is referred to as phase 1 of the simulation. During the period from 1901-1914 (phase 2), the total population size was limited to 1000 individuals and male calves were additionally culled to achieve a 5:1 female:male sex ratio after the population reached 100 individuals.

In 1914, the Custer State Park (CSP) population was formed from individuals taken from the Philip population (Appendix B). This is the beginning of phase 3 of the simulation. The mature females used to found CSP were assumed to be between two and eight years of age. The mature males were assumed to be between four and eight years of age, and the 12 calves were assumed to include nine females and three males, as this was the sex ratio of the mature animals selected to found the CSP population. In the simulations, individuals used to found the CSP population were randomly sampled from the appropriate age and sex categories found in the Philip population. The CSP population is currently managed to keep a 7:1 female:male sex ratio (Walker 1998). In the simulations, males were culled as described above, in order to achieve a 7:1 female:male sex ratio, and the maximum population size was limited to 3000 individuals.

Fecundity of Female North American Bison

Female North American bison first reproduce at three years of age, and produce an average of 0.7 calves per year through the age of 13 years. From 14 years of age until death, they produce an average of 0.3 calves per year. Variation in breeding success within these age classes is not significant. This information has been adapted from Berger and Cunningham (1994).

Fecundity of Male North American Bison

Variation in male reproductive success is characterized by differences in reproductive success for males in different age categories, and by life long differences between males within the same age category. In the simulations, the total reproductive output for each age class is given below. In a given year, the four and five year old male age classes will each sire 1% of the calves. The six year old age class will sire 5% of the calves. The seven through 12 year old age classes will each sire 12.7% of the calves, and the 13 through 15 year old age classes will sire 5.6% of the calves. After 15 years of age, males do not make a significant contribution to reproduction, and are culled from the population in the simulations. Variance in reproductive success within an age class is accounted for by assigning breeding values to males at birth. Half of the males born in a given year are assigned a breeding value of 1, 20% are assigned a breeding value of 3, and 10% each, are assigned breeding values of 5, 7, and 8. Before each round of mating, the relative breeding potential of each male is determined by dividing his breeding potential by the sum of the breeding potentials for all of the males in his age group. The number of

calves sired by a given male each year is the product of his relative breeding potential and the total reproductive output for his age group. In this way, age-related and life long variance in breeding success among males is taken into consideration. The values used in the determination of variance in male reproductive success have been adapted from Berger and Cunningham (1994).

Viability, Fertility, and Fecundity of Hybrids

Crosses between pure North American bison and domestic cattle, produced viable female offspring 26.5% of the time (Boyd 1908, 1914). The rare production of a viable male offspring was ignored because all F1 males are completely sterile (Jones 1907; Boyd 1908, 1914; Goodnight 1914; Gray 1954), and would therefore not contribute to the population. Female F1 hybrids produce an average of 0.125 calves per year from the time they are three years old until they are 13 years old, at which time they effectively produce no further calves (Boyd 1908, 1914). Crosses involving F1 females and male North American bison or domestic cattle produce a 60:40 female:male sex ratio (Boyd 1908, 1914). Females resulting from this cross will be completely fertile, while only 25% of the viable males resulting from such a cross will be fertile (Boyd 1908, 1914). All further backcross generations are assumed to exhibit normal viability and fertility in both sexes.

Mortality

Mortality was set at 6% per year for males, and 4% per year for females from birth through two years of age. From three through 14 years of age, mortality was set at 3% per year in both sexes. After 14 years of age, mortality was set to 50% per year for both sexes, with males being culled from the population at 16 years of age, and females being culled from the population at 20 years of age. These values were adapted from Peterson (1990).

APPENDIX D

LETTER OF PERMISION

CAMBRIDGE UNIVERSITY PRESS

PUBLICATION PERMISSION

May 20, 1999

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Reference

Journal: *Animal Conservation*, Vol. 2, (1999):51-57

Author: Ward, Todd J.

Title: "Identification of domestic cattle hybrids in wild cattle and bison species: A general..."

Selection/pp.: entire article

Additional:

Use

Reprint Title: dissertation

Publisher: Texas A&M University

Format/Qty: dissertation

Avail. Date: 1999

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Department of Biological Sciences	June, 1995

Publications

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