Genetic relationship of wood and plains bison based on restriction fragment length polymorphisms

A. M. Bork[1]
Department of Animal Science, University of Alberta, Edmonton, Alta., Canada T6G 2P5

C. M. Strobeck
Department of Zoology, University of Alberta, Edmonton, Alta., Canada T6G 2P5

F. C. Yeh
Department of Forest Science, University of Alberta, Edmonton, Alta., Canada T6G 2P5

and

R. J. Hudson and R. K. Salmon
Department of Animal Science, University of Alberta, Edmonton, Alta., Canada T6G 2P5

Received August 21, 1989

Bork, A. M., Strobeck, C. M., Yeh, F. C., Hudson, R. J., and Salmon, R. K. 1991. Genetic relationship of wood and plains bison based on restriction fragment length polymorphisms. Can. J. Zool. **69**: 43–48.

To evaluate the genetic relationship within and between wood and plains bison of Elk Island National Park, genomic DNA samples were screened for restriction fragment length polymorphisms (RFLP) with cDNA probes for growth hormone, growth hormone releasing factor, somatostatin, and insulin-like growth factor-1. Of the 28 fragments identified, two revealed RFLPs, both of which were associated with the growth hormone releasing factor locus. The observed frequencies of the polymorphic sites did not differ from a Hardy–Weinberg distribution in either population, which is indicative of random mating populations. The contingency χ^2 tests for homogeneity indicate that the fragment frequencies of the polymorphic restriction sites differ significantly ($p = 0.00$) between the wood and plains bison. The number of net nucleotide substitutions between the two populations was 0.0007, indicative of a recent divergence. Conversion of the bison nucleotide divergence results in a relative protein divergence of 0.007 to 0.018. This converted divergence corresponds closely to the divergence reported for other geographically isolated populations; thus, this preliminary analysis suggests the bison have at least reached the stage of geographic isolation in their evolutionary divergence.

Bork, A. M., Strobeck, C. M., Yeh, F. C., Hudson, R. J., et Salmon, R. K. 1991. Genetic relationship of wood and plains bison based on restriction fragment length polymorphisms. Can. J. Zool. **69** : 43–48.

Nous avons évalué la relation génétique au sein des populations de bisons des bois et de bisons des plaines d'une part, et entre ces populations d'autre part, dans le parc national d'Elk Island; des sondes d'ADN complémentaire capables de repérer l'hormone de croissance, le facteur de libération de l'hormone de croissance, la somatostatine et le facteur-1 de croissance semblable à l'insuline ont servi à retrouver les polymorphismes des fragments de restriction (RFLP) dans des échantillons d'ADN génomique. Des 28 fragments identifiés, deux étaient le siège de RFLPs, tous deux associés au locus du facteur de libération de l'hormone de croissance. Les fréquences observées des sites de polymorphisme correspondaient à une distribution Hardy–Weinberg dans les deux populations, ce qui reflète un système où les accouplements se font au hasard. Les tests de contingence du χ^2, destinés à mesurer l'homogénéité, ont montré que la fréquence des fragments aux sites des restrictions polymorphes différait significativement ($P = 0,00$) chez les bisons des plaines et les bisons des bois. Le nombre de substitutions nettes de nucléotides entre les deux populations était de 0,0007, ce qui reflète une divergence récente. La conversion de cette divergence des nucléotides résulte en une divergence relative de protéines de 0,007 à 0,018. Cette divergence convertie correspond étroitement à la divergence constatée chez d'autres populations isolées géographiquement; cette analyse préliminaire indique donc que les deux types de bisons ont atteint au moins le stade d'isolement géographique dans leur divergence évolutive.

[Traduit par la revue]

Introduction

The existence of a distinct form of bison in northern Canada was recognized over 100 years ago and eventually culminated in the designation of this northern form as the subspecies *Bison bison athabascae*, commonly known as the wood bison (van Zyll de Jong 1986). However, the lack of an unambiguous concept of a subspecies has resulted in a persistent controversy concerning the validity of this subspecific designation as distinct from the plains bison (*Bison bison bison*) (Peden and Kraay 1979; McDonald 1981; van Zyll de Jong 1986). Wood bison are usually distinguished from plains bison by their larger body parameters, larger horn cores, dark woolly pelage, and a relatively small amount of hair on their upper forelegs and beard (Geist and Karsten 1977; Reynolds *et al.* 1982). Using multivariate morphometrics to analyze body size parameters, van Zyll de Jong (1986) considered that the phenotypic discontinuity in body size parameters fully justified the subspecific distinctions. However, the conclusion of van Zyll de Jong (1986) is inconsistent with the previous observations of Peden and Kraay (1979) who found that the blood types and carbonic anhydrase polymorphisms in wood, plains, and wood × plains hybrid bison were similar. On the basis of their study, Peden and Kraay (1979) argued that the classification of wood and plains bison as separate subspecies could be questioned. Other comparisons of the blood types and karyotypes of wood and plains bison (Ying and Peden 1977; Zamora 1983) have been no more successful in clarifying the taxonomic relationships between the two bison types.

Several recent studies have revealed the potential of restriction endonuclease analysis of mitochondrial DNA to clarify the genetic relationships and evolutionary histories of various groups of organisms (e.g., Avise *et al.* 1979*b*; Ferris *et al.* 1983*a*; Ferris *et al.* 1983*b*; Powell 1983; Wright *et al.* 1983; Spolsky and Uzzell 1986). However, preliminary work with

[1]Author to whom correspondence should be addressed.

wood and plains bison mitochondrial DNA has been inconclusive as additional comparisons between the subspecies are required to establish criteria for classification (Cronin 1986).

In a restriction site analysis of mice with divergent growth patterns, Salmon *et al.* (1988) reported that genetic variation at the growth hormone gene locus was strongly associated with body size. Since body size parameters are used to differentiate between wood and plains bison, variation may exist at growth-regulating genes within the two bison types. Therefore, the study reported herein employed restriction endonuclease analysis of four genes (growth hormone (GH), growth hormone releasing factor (GHRF), somatostatin (S), and insulin-like growth factor-1 (IGF-1)) known to regulate postnatal mammalian growth (Raisz and Kream 1981; Froesch *et al.* 1985; Zapf and Froesch 1986; Gorbman *et al.* 1983) to explore the genetic relationships between the two North American bison types.

Materials and methods

Sample collection

Approximately 10 mL of blood was drawn from the tail vein of 40 plains bison and 40 wood bison randomly selected from the two bison populations maintained in Elk Island National Park (EINP). The blood was collected in EDTA vacutainers (Beckton Dickinson and Company, Rutherford, NJ) and immediately stored between 0 and 4°C (wet ice or refrigerator) until DNA extraction.

Four European bison (wisent) samples were obtained for comparison with polymorphic loci discovered between the two North American bison subspecies. Three wisent DNA samples, which originated in Poland, were generously provided by M. Fellous (Institut Pasteur, France). The fourth wisent blood sample was provided by C. G. Penny (San Diego Zoo, CA).

DNA preparation

To facilitate the extraction of DNA, white blood cells were isolated and subsequently lysed. To isolate white blood cells, five volumes of a 0.155 M NH_4 Cl – 0.17 M Tris solution (prewarmed to 37°C) was added to one volume of whole blood. Following a 5-min incubation at 37°C, the solution was centrifuged at 2000 rpm (Beckman JA-20 rotor) for 10 min. After aspiration of the supernatant, the pellet was resuspended in 10 mL of a 0.85% NaCl solution and centrifuged at 2000 rpm for 10 min. This sequence of aspiration, resuspension in 0.85% NaCl, centrifugation, and aspiration was then repeated. The white blood cells in the final pellet were lysed by resuspending this pellet in 2 mL of 100 mM Tris (pH 8.0) containing 1 mM EDTA and then immediately injecting 2 mL of 100 mM Tris (pH 8.0) containing 40 mM EDTA and 1.2% SDS.

For the extraction of DNA, an equal volume of phenol saturated with TE (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) was added to the solution of lysed cells and mixed for 10 min. The resulting emulsion was then centrifuged at 5000 rpm (Beckman JA-20 rotor) for 5 min. After centrifugation, the upper aqueous phase was reextracted with an equal volume of TE-saturated phenol. This second phenol extraction was followed by an extraction with an equal volume of 1:1 phenol–chloroform and, subsequently, with an equal volume of 24:1 chloroform – isoamyl alcohol. The final aqueous phase was then dialysed against three changes of TE over a 24-h period. Following dialysis, DNA was ethanol precipitated and resuspended in TE.

A total of 10 commercially obtained restriction endonucleases (Bethesda Research Laboratories, Burlington, Ont.; Pharmacia, Dorval, Que.) were used to digest the bison DNAs. Addition of the restriction enzyme was carried out over 6 h and total digestion time ranged between 18 and 22 h. The digested DNAs were electrophoresed on 0.7% agarose gels in 0.04 M Tris-acetate buffer containing 1 mM EDTA (Maniatis *et al.* 1982) at 30 V for 20–24 h. Ethidium bromide (0.3 μg/mL) was added to the gel to allow visualization of DNA. Each gel included DNA markers of known fragment sizes: bacteriophage λ cleaved with *Hin*dIII, pBr322 cleaved with *Sau*3AI, as well as high molecular weight (HMW) marker (BRL). The size of the marker fragments ranged from 48 to 0.36 kb to allow for an estimation of bison fragment size. After electrophoresis, the digested DNA fragments were transferred onto a nylon membrane (GeneScreen Plus, New England Nuclear Research Products, Boston, MA). Treatment of the DNA before and after transfer to the membrane followed the conditions recommended by the supplier. The method of transfer followed the protocol outlined in Maniatis *et al.* (1982). Transfer was carried out for 38–48 h.

The four cDNAs (complementary DNAs) used for hybridization analysis were bovine growth hormone (bGH/pSP65) (Gordon *et al.* 1983), human somatostatin (pHS/pRTB1-63) (Shen *et al.* 1982), human insulin-like growth factor-I (hIGF-1B/pGEM1) (Rotwein 1986), and rat growth hormone releasing factor (hGRF/λ101) (Mayo *et al.* 1985). Plasmids containing the above cDNA were transformed into appropriate host bacterial strains, isolated, and purified following the procedures outlined in Maniatis *et al.* (1982). Inserts were cleaved from plasmids by restriction enzyme digestion and separated from vector DNA and LMP agarose (BRL) gels. Probes were radiolabelled with [^{32}P]dCTP (ICN) by the random primer method (Feinberg and Vogelstein 1983, 1984). The labelled probe fragments were separated from the unincorporated radionucleotides by spun-column chromatography (Maniatis *et al.* 1982). Prehydridization, hybridization, and washing of the membrane followed the formamide procedure recommended by the supplier. Membranes were autoradiographed at −70°C using two sheets of Kodak GBX-2 film (Eastman Kodak Co., Rochester, NY) and Lightning Plus intensifying screens (Dupont). Films were exposed for 3–14 days depending on probe activity.

Statistical analysis

The fragments, identified via autoradiography, were considered in terms of restriction site locality. Although more complex sequence alterations could undoubtedly occur, the assumption was made that a dimorphic restriction fragment reflected the existence of a single dimorphic restriction site.

The genotypic frequencies for the wood and plains bison populations were calculated for each polymorphic site identified and were compared to Hardy–Weinberg proportions. The differences between the observed and expected values were analyzed using χ^2 tests corrected for small sample size (Levene 1949). The extent of homogeneity of fragment frequencies for each polymorphic site between the bison populations was estimated through the contingency χ^2 analysis (Feinberg 1983).

DNA divergence between the two subspecies was determined through Nei's (1987) formula. The number of net nucleotide substitutions (DNA divergence) between two populations (D) is estimated by

$$[1] \quad D = D_{xy} - (D_x + D_y)/2$$

where D_x is the average number of nucleotide substitutions for DNA haplotypes in population x, D_y is the average number of nucleotide substitutions for haplotypes in population y, and D_{xy} is the average number of nucleotide substitutions between DNA haplotypes from populations x and y. D_x, D_y, and D_{xy} were calculated using equation 5.42 (Nei 1987, p. 101) and equations 10.19 and 10.20 (Nei 1987, p. 276). The haplotype frequencies used for this calculations are given in Table 1.

Results

The 22 enzyme–probe combinations used in this study provided 28 fragments (50 restriction sites) for the examination of intra-and inter-population genetic variability (see Table 2). Of these 28 fragments, two revealed restriction fragment length polymorphisms (RFLPs), both of which were associated with the GHRF locus. When digested with *Eco*RV, the bison GHRF locus yielded fragments that were either 8.9 or 14.4 kb; *Sph*I digestion of this locus yielded fragments that were either 11.1 or 12.6 kb in length (Fig. 1). Each of these RFLPs was identified within both the wood and plains bison samples. These fragments were interpreted as diallelic polymorphisms at each of

TABLE 1. Observed genotypes at *Sph*I and *Eco*RV polymorphic sites

	*Sph*I		
*Eco*RV	S_1S_1	S_1S_2	S_2S_2
E_1E_1	10 wood	0	0
E_1E_2	4 wood 3 plains	6 wood 5 plains	0
E_2E_2	1 plains	7 plains	4 plains

NOTE: Frequencies of haplotypes: $E_1S_1 = 38$, $E_1S_2 = 0$, $E_2S_1 = 16$, and $E_2S_2 = 26$. Since no single or double homozygotes involving E_1S_2 haplotype were observed, it is assumed the "double heterozygotes" consist of haplotypes E_1S_1 and E_2S_2.

TABLE 2. Restriction fragment lengths associated with growth-regulating loci

Enzyme	Locus	Fragment sizes (kb) Allele 1	Allele 2	No. of restriction sites
		Wood–plains bison		
*Eco*RI	GH	5.40		2
	IGF-1	9.80		4
		8.20		
		3.45		
	S	18.90		3
		3.20		
	GHRF	5.65		2
*Bam*HI	GH	13.90		2
	IGF-1	15.10		3
		13.00		
	GHRF	14.00		2
*Pst*I	GH	.64		2
	IGF-1	6.40		3
		5.90		
	GHRF	3.81		2
*Hin*dIII	GH	20.50		2
	S	20.54		3
		8.00		
	GHRF	8.75		2
*Pvu*II	GH	1.00		2
	S	21.00		2
*Xha*I	GH	13.70		2
	S	8.70		2
	GHRF	3.85		2
*Bgl*I	GHRF	7.00		2
*Dra*I	GHRF	6.95		2
*Eco*RV	GHRF	8.90 (E_1)	14.40 (E_2)	2
*Sph*I	GHRF	11.10 (S_1)	12.60 (S_2)	2
Total				50
		European bison		
*Eco*RV	GHRF	14.40		
*Sph*I	GHRF	11.10		

two loci; individual bison could be either homozygous or heterozygous for each of the *Eco*RV and *Sph*I fragments.

There was evidence for differentiation of fragment frequencies between wood and plains bison (Tables 3,4). The contingency χ^2 tests for homogeneity of fragment frequencies between the bison populations indicated divergence at both *Eco*RV

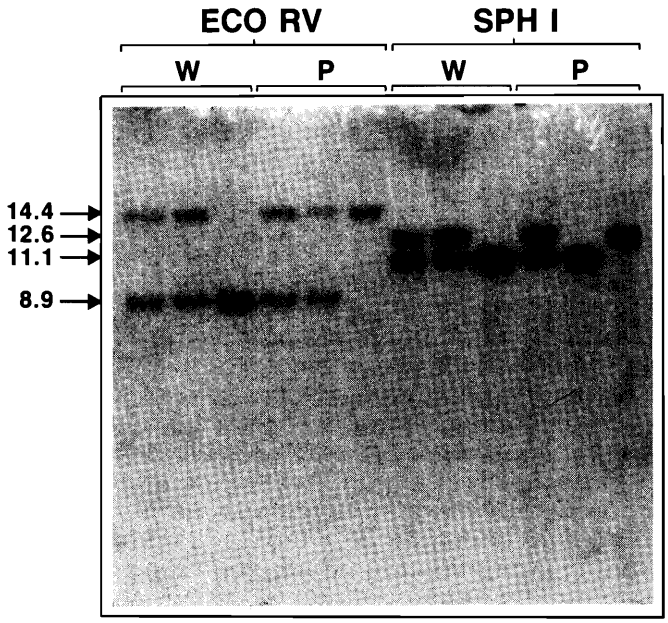

FIG. 1. Autoradiogram of the GHRF-related RFLPs within the wood (W) and plains (P) bison (for methodology, refer to Materials and methods). These polymorphic restriction fragments were revealed when bison DNA was digested with either *Eco*RV or *Sph*I. The size of the fragments are shown in kilobases on the left margin; *Sph*I fragments are 11.1 and 12.6 kb, the *Eco*RV fragments are 8.9 and 14.4 kb. Individual wood and plains bison could be either homozygous (single, dark-staining band) or heterozygous (two bands) for each of the *Eco*RV and *Sph*I restriction fragments.

TABLE 3. Frequency of alleles at polymorphic loci in wood and plains bison

		Population	
Enzyme	Locus	Wood ($N = 20$)	Plains ($N = 20$)
*Eco*RV	E_1	0.75	0.20
	E_2	0.25	0.80
*Sph*I	S_1	0.85	0.50
	S_2	0.15	0.50

($\chi^2 = 24.26$, 1 df, $p = 0.0$) and *Sph*I ($\chi^2 = 11.17$, 1 df, $p = 0.0$). Within wood bison, E_1 appeared at a higher frequency than E_2, whereas the reverse was true within the plains bison. At the dimorphic *Sph*I site, S_1 appeared at a higher frequency than S_2 within wood bison, whereas S_1 and S_2 appeared at equal frequency within the plains bison. The number of net nucleotide substitutions (DNA divergence) between the two populations was 0.0007.

The expected and observed genotypic frequencies at dimorphic sites within the experimental animals are presented in Table 4. The observed frequencies of the dimorphic sites did not differ from the Hardy–Weinberg expectations within either the wood or the plains bison.

A comparison of the North American bison GHRF-related restriction fragments with those identified in the four European bison samples is presented in Fig. 2. Both *Eco*RV and *Sph*I identify GHRF-related restriction fragments that were also identified within the North American species. The European bison *Eco*RV restriction fragment is 14.4 kb and the *Sph*I

TABLE 4. χ^2 test for deviation from Hardy–Weinberg equilibrium

Enzyme	Genotype	Frequency Observed	Frequency Expected	χ^2	df	p
		Wood bison				
*Eco*RV	E_1E_1	10	11.15			
	E_1E_2	10	7.69			
	E_2E_2	0	1.15	1.97	1	0.16
*Sph*I	S_1S_1	14	14.39			
	S_1S_1	6	5.23			
	S_2S_2	0	0.39	0.51	1	0.48
		Plains bison				
*Eco*RV	E_1E_1	0	0.72			
	E_1E_1	8	6.56			
	E_2E_2	12	12.72	1.07	1	0.30
*Sph*I	S_1S_1	4	4.87			
	S_1S_2	12	10.26			
	S_2S_2	4	4.87	0.61	1	0.44

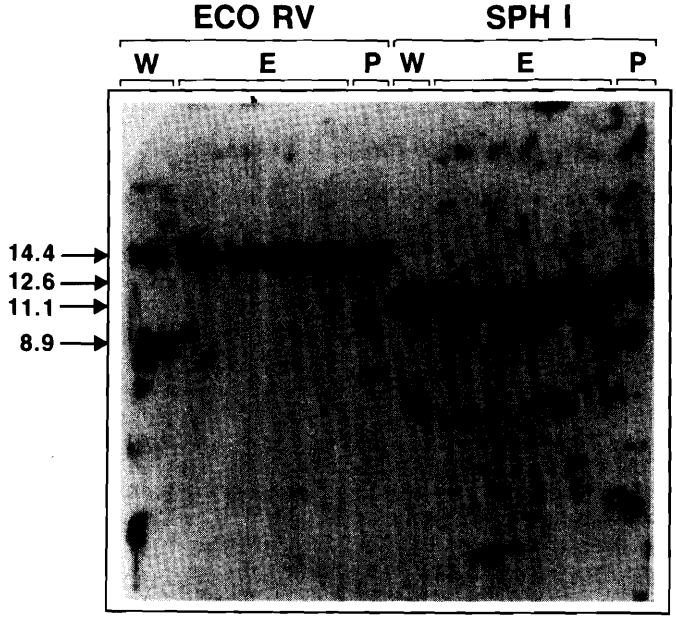

FIG. 2. Autoradiogram of the European (E) and North American (wood (W) and plains (P)) bison GHRF-related restriction fragments (for methodology, refer to Materials and methods). The size of the fragments are shown in kilobases in the left margin. When European bison DNA was digested with *Eco*RV, the GHRF locus yielded a restriction fragment of 14.4 kb, and digestion with *Sph*I yielded a fragment of 11.1 kb.

restriction fragment is 11.1 kb. The discovery of these fragments within the wisent implies that they are of primitive origin while the other restriction fragments (*Eco*RV 8.9 kb and *Sph*I 12.6 kb) discovered within the North American bison are the derived fragments (outgroup rule) (Wiley 1981, p. 139). It would have been beneficial to examine the wisent with the other 20 probe–enzyme combinations; however, analysis was restricted as a result of limited amounts of wisent DNA.

Discussion

Intrapopulation variability

As defined by restriction site loci associated with growth-regulating genes, the genetic diversity within both the wood and plains bison populations at EINP appears to be low; polymorphisms were identified at only 2 of the 50 restriction site loci examined. This low level of intrapopulation genetic diversity is compatible with the history of the two populations; in the establishment of both the plains and the wood population, a very limited number of animals were available. The bison populations that exist today result from an original stock of 22 wood bison and 30 plains bison (Ogilvie 1979; Novakowski 1989). Since the populations are derived from a limited genetic foundation, the 20 animals sampled in this study probably provide a good indication of the variability present within bison.

While very few restriction site polymorphisms were identified within the wood and plains bison, the distribution of the identified polymorphic sites does reveal several structural features of the existing EINP populations. The Hardy–Weinberg equilibrium of the restriction site genotypes, in the wood and plains bison sampled (Table 4), implies that both bison populations are random mating; each male in the population appears to have an equal chance of mating with each female. Thus, although both populations have experienced bottlenecks in the past, our study suggests that the existing genetic information within each population is being evenly distributed.

Deviations from Hardy–Weinberg equilibrium may result if the allele frequencies in the small number of bulls mating differ from the allele frequencies in females (owing to sampling error). Therefore, the concept of random mating within each bison population may appear to be incompatible with the polygynous mating system typical of bison (Lott *et al.* 1987). The principal factor influencing this random distribution of genotypes is time. Both bison populations in EINP were established over 25 years ago, and this span encompasses the predominant lifetime of a bison. Within the polygynous mating system, selection for body size, weaponry, and behavior in males restricts monopolization of females to certain age classes (Jarman 1983). Although the age at which male bison may breed is a selective factor, over a lifetime most bulls will eventually mate, thus facilitating random mating within the bison populations.

Population structure of the EINP bison may also contribute to the randomized arrangement of genotypes. With the exception of the breeding season, bison bulls remain either solitary or roam in small temporary groups; cows form larger groups with the calves and young bulls. As the mating season approaches, the cows congregate into larger breeding clusters where they are joined by the mature bulls (Lott 1972, 1974, 1979). Therefore, depending upon the size of the breeding cluster relative to the total population size, a small number of dominant bulls may have access to a large proportion of the breeding females; the inability of many less dominant bulls to contribute to the gene pool could thereby result in genotypic frequencies which deviate from a Hardy–Weinberg equilibrium. However, Shackleton (1968) indicated that the group size of bison, within the wooded habitat of EINP, is smaller than it would be in open range. This smaller group size would increase the number of breeding clusters available. As a consequence, the less dominant bulls would have a greater probability to contribute to the gene pool, thereby increasing the opportunity to distribute genetic material uniformly throughout the population.

Random distribution of genotypes may also be attributed to the mobility of cows. Individual females are known to move rapidly and extensively through home ranges (Lott and Minta 1983). This thorough population mixing would increase each cow's chances of encountering different bulls in various years. Moreover, since the bulls are not associated with a specific herd

throughout much of the year, further distribution of available genetic material may result through the random distribution of bulls among the breeding clusters during each breeding season.

Interpopulation variability

The contingency χ^2 analysis indicates that the fragment frequencies of the identified polymorphic restriction sites differ significantly between the two populations. This significant difference is compatible with the suggestion that the wood and plains bison exist as two unique populations.

Although considerable controversy exists regarding both the taxonomic status of wood and plains bison as well as their evolutionary history (for review see McDonald 1981), a consensus does exist that wood and plains bison evolved from a common ancestor (Wilson 1969; Guthrie 1970; Geist and Karsten 1977; McDonald 1981; van Zyll de Jong 1986). The time of divergence of the two bison types has been estimated by Wilson (1969) to have occurred approximately 5000 years ago.

The level of genetic divergence (D = 0.0007) between the two bison populations indicates a recent time of divergence. Nucleotide divergence data for geographically isolated populations are scarce in comparison with the more extensive research of protein analysis for population differentiation. However, comparison of nucleotide-based analysis with protein-based analysis allows only very general conclusions to be drawn.

Genetic divergence of Hawaiian *Drosophila* species have been calculated on the basis of protein analysis as well as nucleotide analysis (Bishop and Hunt 1988). This study revealed 10.6 to 25 times greater variation at the protein level than at the nucleotide level. Taking this 10.6- to 25-fold increase into account, the level of nucleotide divergence between the bison populations corresponds to a protein divergence of 0.007–0.018. This converted bison divergence corresponds closely to the divergence discovered in local races (geographically isolated populations) of *Drosophila* (D = 0.008–0.049) and mice (D = 0.010–0.024) (Nei 1987). In terms of large mammals, Gyllensten *et al.* (1983) reported an average divergence of 0.016 for subspecies of red deer (*Cervus elaphus*). It has been suggested that it would be more appropriate to reclassify the red deer as local races (Nei 1987).

Thus, our preliminary restriction site analysis suggests that the wood and plains bison populations at EINP have at least reached the stage of geographic isolation in their evolutionary divergence. The first step of the evolution (speciation) process is the isolation of populations by geographic barriers whereupon each population accumulates unique genetic differences. In the next step of geographic speciation, organisms are recognized as subspecies. At this level, differentiated populations have achieved at least partial reproductive isolation in which matings between individuals result in progeny with reduced fitness (Ayala 1975). According to this definition, the reproductive ability exhibited by the hybrid bison in Wood Buffalo National Park would indicate that the wood and plains bison have not yet obtained this stage in their evolutionary divergence. However, according to the guidelines of the Committee on the Status of Endangered Wildlife in Canada (1979), this classification of wood and plains bison as geographic populations would require that separate management of the two bison types be continued.

A considerable expansion of our molecular analysis of the wisent will be very valuable in further defining the taxonomic status of the wood and plains bison. While the actual time of divergence between the European bison and the North American bison remains uncertain (Wilson 1969; Guthrie 1970; Geist and Karsten 1977; McDonald 1981), the wisent has been geographically separated for at least 10 000 years (McDonald 1981). Fluctuations in sea level, caused by the freezing and thawing of glaciers, resulted in the appearance and disappearance of a land connection between eastern Siberia and Alaska. This land bridge made possible the migration of the European bison into North America; the Bering Land Bridge last joined Siberia and Alaska during the Late Wisconsin period (McDonald 1981). On the basis of this geographic separation, and on the significant phenotypic differences between the European and North American bison (van Zyll de Jong 1986), most researchers (for review see McDonald 1981) appear confident in their classification of the wisent as a distinct species. An expanded examination of the nucleotide variation, which exists among these three bison types, would allow their degree of relationship with one another to be predicted; the degree of relatedness between wood and plains bison could then be considered relative to the relatedness between the North American and European bison forms. This further molecular analysis of wood, plains, and wisent populations, as well as of populations with clearly defined subspecific relationships, will undoubtedly be necessary to substantiate the subspecific designations of wood and plains bison.

Acknowledgements

We extend our sincere appreciation to C. Blyth (Parks Canada), C. M. Gates (Government of the Northwest Territories), and H. W. Reynolds (Canadian Wildlife Service) for their enthusiastic support of this project. We also gratefully acknowledge the invaluable cooperation of Parks Canada personnel at EINP during the collection of blood samples. Financial support for this project was provided, in part, by the Department of Renewable Resources, Government of the N.W.T.

AVISE, J. C., LANSMAN, R. A., and SHADE, R. O. 1979. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. I. Population structure and evolution in the genus *Peromyscus*. Genetics, **92**: 279–295.

AYALA, F. J. 1975. Genetic differentiation during the speciation process. Evol. Biol. **8**: 1–78.

BISHOP, J. C., and HUNT, J. A. 1988. DNA divergence in and around the alcohol dehydrogenase locus in five closely related species of Hawaiian *Drosophila*. Mol. Biol. Evol. **5**: 415–431.

COMMITTEE ON THE STATUS OF ENDANGERED WILDLIFE IN CANADA. 1979. Endangered Canadian Wildlife. Summary Sheets. Canadian Wildlife Service, Edmonton.

CRONIN, M. A. 1986. Genetic relationships between white-tailed deer, mule deer and other large mammals inferred from mitochondrial DNA analysis. M.Sc. thesis, Montana State University, Bozeman.

FEINBERG, A. P., and VOGELSTEIN, B. 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**: 6–13.

——— 1984. Addendum to a technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **137**: 266–267.

FEINBERG, S. E. 1983. The analysis of cross-classified categorical data. 2nd ed. The MIT Press, Cambridge.

FERRIS, S. D., SAGE, R. D., PRAGER, E. M., RITTE, U., and WILSON, A. C. 1983*a*. Mitochondrial DNA evolution in mice. Genetics, **105**: 681–721.

FERRIS, S. D., SAGE, R. D., HUANG, C. M., NEILSON, J. T., RITTE, U., and WILSON, A. C. 1983*b*. Flow of mitochondrial DNA across a species boundary. Proc. Natl. Acad. Sci. U.S.A. **80**: 2290–2294.

FROESCH, E. R., SCHMID, C., SCHWANDER, J., and ZAPF, J. 1985. Actions of insulin-like growth factors. Annu. Rev. Physiol. **47**: 443–467.

GEIST, V., and KARSTEN, P. 1977. The wood bison (*Bison bison athabascae* Rhoads) in relation to hypotheses on the origin of the

American bison (*Bison bison* Linnaeus). Z. Saeugetierkd. **42**: 119–127.

GORBMAN, A., DICKHOFF, W. W., VIGNA, S. R., CLARK, N. B., and RALPH, C. L. 1983. Comparative endocrinology. John Wiley and Sons, New York. pp. 125–136, 547–558.

GORDON, D. F., QUICK, D. P., ERWIN, C. R., DONELSON, J. E., and MAURER, R. A. 1983. Nucleotide sequence of the bovine growth hormone chromosomal gene. Mol. Cell. Endocrinol. **33**: 81–95.

GUTHRIE, R. D. 1970. Bison evolution and zoogeography in North America during the Pleistocene. Q. Rev. Biol. **45**: 1–15.

GYLLENSTEN, U., RYMAN, N., REUTERWALL, C., and DRATCH, P. 1983. Genetic differentiation in four European subspecies of red deer (*Cervus elaphus L.*). Heredity, **51**: 61–80.

JARMAN, P. 1983. Mating system and sexual dimorphism in large, terrestrial, mammalian herbivores. Biol. Rev. **58**: 485–520.

LEVENE, H. 1949. On a matching problem arising in genetics. Ann. Math. Stat. **20**: 91–94.

LOTT, D. F. 1972. Bison would rather breed than fight. Nat. Hist. **81**: 40–45.

——— 1974. Sexual and aggressive behavior of mature male American bison. *In* Behavior in ungulates and its relation to management. *Edited by* V. Geist and F. Walthers. IUCN, Morges. pp. 382–394.

——— 1979. Dominance relations and breeding rate in mature male American bison. Z. Tierpsychol. **49**: 418–432.

LOTT, D. F., and MINTA, S. C. 1983. Random individual association and social group instability in American bison (*Bison bison*). Z. Tierpsychol. **61**: 153–172.

LOTT, D. F., SHAW, J. H., and STORMONT, C. 1987. Should public herds be trading bison to maintain diversity in the gene pool and/or prevent inbreeding depression? *In* Proceedings of the North American Bison Workshop, Sponsored by United States Fish and Wildlife and Glacier Natural History Association, Missoula, MT. pp. 59–60.

MANIATIS, T., FRITSCH, E. F., and SAMBROOK, J. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor.

MAYO, E., CERELLI, G. M., ROSENFELD, M. G., and EVANS, R. M. 1985. Characterization of cDNA and genomic clones encoding the precursor: to rat hypothalamic growth hormone-releasing factor. Nature (London), **314**: 464–467.

MCDONALD, J. N. 1981. North American bison, their classification and evolution. University of California Press, Berkeley.

NEI, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

NOVAKOWSKI, N. S. 1989. Historical origin of national park plains bison. Department of the Environment, Canadian Park Service, Natural Resources Branch, Ottawa.

OGILVIE, S. C. 1979. The park buffalo. Calgary–Banff Chapter. National and Provincial Park Association, Canada.

PEDEN, D. G., and KRAAY, G. J. 1979. Comparison of blood characteristics in plains bison, wood bison, and their hybrids. Can. J. Zool. **57**: 1778–1784.

POWELL, J. R. 1983. Interspecific cytoplasmic gene flow in the absence of nuclear gene flow: evidence from *Drosophila*. Proc. Natl. Acad. Sci. U.S.A. **80**: 492–495.

RAISZ, L. G., and KREAM, B. E. 1981. Hormonal control of skeletal growth. Annu. Rev. Physiol. **43**: 225–238.

REYNOLDS, H. W., GLAHOLT, R. D., and HAWLEY, A. W. L. 1982. Bison. *In* Wild mammals of North America. *Edited by* J. A. Chapman and G. A. Feldhamer. The Johns Hopkins University Press, Baltimore. pp. 972–1007.

ROTWEIN, P. 1986. Two insulin-like growth factor I messenger RNAs are expressed in human liver. Proc. Natl. Acad. Sci. U.S.A. **83**: 77–81.

SALMON, R. K., BERG, R. T., YEH, F. C., and HODGETTS, R. 1988. Identification of a variant growth hormone haplotype in mice selected for high body weight. Genet. Res. **52**: 7–15.

SHACKLETON, D. M. 1968. Comparative aspects of social organization of American bison. M. Sc. thesis, University of Western Ontario, London.

SHEN, L., PICTET, R. L., and RUTTER, W. J. 1982. Human somatostatin I: sequence of the cDNA. Proc. Natl. Acad. Sci. U.S.A. **79**: 4575–4579.

SPOLSKY, C., and UZZELL, T. 1986. Evolutionary history of the hybridogenetic hybrid frog *Rana esculenta* as deduced from mtDNA analysis. Mol. Biol. Evol. **3**: 44–56.

VAN ZYLL DE JONG, C. G. 1986. A systematic study of recent bison, with particular consideration of the wood bison. National Museums of Canada, Publications in Natural Sciences No. 6.

WILEY, E. O. 1981. Phylogenetics. The theory and practice of phylogenetic systematics. John Wiley and Sons, New York.

WILSON, W. 1969. Problems in the speciation of American fossil bison. *In* Post-Pleistocene man and his environment on the Northern Plains. Proceeding of the First Annual Paleo-environmental Workshop. Students' press, University of Calgary, Calgary.

WRIGHT, J. W., SPOLSKY, C., and BROWN, W. M. 1983. The origin of the parthenogenic lizard *Cnemidophorus laredoensis* inferred from mitochondrial DNA analysis. Herpetologica, **39**: 410–416.

WRIGHT, S. 1931. Evolution in Mendelian populations. Genetics, **16**: 97–159.

YING, K. L., and PEDEN, D. G. 1977. Chromosomal homology of wood bison and plains bison. Can. J. Zool. **55**: 1759–1762.

ZAMORA, L. E. 1983. An analysis of bison erythrocyte antigens and blood proteins. M. Sc. thesis, Texas A & M University, Betram.

ZAPF, J., and FROESCH, E. R. 1986. Insulin-like growth factors/somatomedins: structure, secretion, biological actions and physiological role. Horm. Res. **24**: 121–130.