Genetic diversity among Canadienne, Brown Swiss, Holstein, and Jersey cattle of Canada based on 15 bovine microsatellite markers

C. Hansen, J.N.B. Shrestha, R.J. Parker, G.H. Crow, P.J. McAlpine and J.N. Derr

Abstract: The genetic diversity among Canadienne, Brown Swiss, Holstein, and Jersey cattle was estimated from relationships determined by genotyping 20 distantly related animals in each breed for 15 microsatellites located on separate chromosomes. The Canadienne, Holstein, and Jersey cattle had an average of six alleles per loci compared with five alleles for Brown Swiss. Furthermore, a number of potentially breed-specific alleles were identified. The allele size variance among breeds was similar, but varied considerably among loci. All of the loci studied were equally heterozygous, as were Brown Swiss, Canadienne, and Holstein cattle (0.68–0.69) whereas Jersey cattle showed lower heterozygosity (0.59). The within-breed estimates of genetic distance were greater than zero and significant. The genetic distance between Canadienne and Holstein (0.156), Brown Swiss (0.243), and Jersey (0.235) was negligible, suggesting close relationship. Concurrently, Brown Swiss and Holstein (0.211) cattle also demonstrated close relationship. In contrast, the Jersey breed was genetically distant from the Brown Swiss and Holstein cattle (0.427 and 0.320, respectively). The characterization of Canadienne cattle, as part of the genetic resource conservation effort currently underway in Canada, underscores the difficulty in scientifically establishing unique breeds. Therefore, the need to consider all relevant morphological characteristics and production performance in combination with available cultural, historical, pedigree, and molecular information becomes relevant when identifying breeds for conservation.

Key words: genetic distance, microsatellites, cattle, genetic resource conservation.

Résumé : La diversité génétique parmi quatre races de bovins (Canadienne, Swiss Brown, Holstein et Jersey) a été évaluée en génotypant 20 animaux peu apparentés chez chacune des races à l'aide de 15 microsatellites situés sur des chromosomes différents. Une moyenne de six allèles par locus a été observée chez les races Canadienne, Holstein et Jersey, tandis que cinq allèles en moyenne étaient observés chez la race Swiss Brown. De plus, certains allèles potentiellement spécifiques d'une race ont été observés. La variance quant à la taille des allèles était semblable d'une race à l'autre, mais variait considérablement d'un locus à un autre. Tous les locus étudiés montraient un niveau semblable d'hétérozygotie, celui-ci variant entre 0,68 et 0,69 chez les races Swiss Brown, Canadienne et Holstein et étant un peu plus faible (0,59) chez la Jersey. La distance génétique mesurée au sein des races était significativement supérieure à zéro. Les distances génétiques entre la Canadienne et soit la Holstein (0,156), la Swiss Brown (0,243) ou la Jersey (0,235) étaient négligeables ce qui suggère une proche parenté. Pareillement, la Swiss Brown et la Holstein montraient également une grande proximité génétique. Par contre, la race Jersey était génétiquement distante de la Swiss Brown et de la Holstein (0,427 et 0,320, respectivement). La caractérisation de la race Canadienne, dans le cadre d'un effort de conservation des ressources génétiques bovines en cours au Canada, montre la difficulté que pose la définition scientifique de races uniques. Ainsi, lorsqu'on tente d'identifier des races à conserver, il importe de considérer tous les caractères morphologiques et la productivité de même que les informations de nature culturelle, historique, généalogique ou moléculaire.

Mots clés : distance génétique, microsatellites, bovins, conservation des ressources génétiques.

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Introduction

In 1601, the ancestors of Canadienne cattle arrived in French Canada with the early settlers, mainly from the Normandy and Brittany regions of France (St-Pierre 1936). Over the years, their relative isolation in what is today the province of Québec, combined with the effects of both natural selection in a harsh environment and creative human activity, resulted in the development of an extremely hardy, self-sufficient breed of small, compact cattle that was well suited to pioneer life (Grisdale 1909). These cattle, known to have played an important role in the colonization of French Canada, not only provided draught power for ploughing and transportation, but also produced both milk and meat for human consumption. In 1883, census results showed that 75% of the cattle in Québec were of the Canadienne breed (Couture 1909). Later, in 1901, Canadienne cattle gained recognition as the most profitable dairy breed at the Pan-American show in Buffalo, N.Y. (Fortin 1940). The trend toward industrial-type milk production, however, created an economic advantage in favour of larger breeds with higher milk production. In an effort to broaden the genetic base and upgrade towards a large-framed milking cow, the Québec Ministry of Agriculture encouraged farmers to crossbreed Canadienne cattle with Brown Swiss bulls. Thereafter, the number of purebreds declined substantially with only 105 Canadienne registered in 1995 (Shrestha and Hansen 1997). The establishment of the milk quota and pricing system in Canada that, until 1992, discriminated against breeds producing milk with a high solids content, further contributed to the decline of the this breed (Bernier 1995). Consequently, Canadienne cattle, under threat of extinction, were accepted as an endangered breed (Hansen 1997; Scherf 2000). Nevertheless, the potential benefit from recent attempts to broaden the genetic base using previously stored semen and embryos appears to be promising (Hansen et al. 2000).

Over the years, biochemical markers, blood groups, allozymes, random amplified polymorphic DNA (RAPD), restriction and amplified fragment length polymorphisms (RFLP and AFLP), and microsatellites (SSRs, simple sequence repeats) have all been used to determine genetic distance based on the frequencies of alleles in various populations (Bretting and Widerlechner 1995; Moazami-Goudarzy et al. 1997). Today, the Food and Agriculture Organization (FAO) and European Association of Animal Production (EAAP) have designated microsatellites as the marker of choice for determining genetic distance. Microsatellite markers, or SSRs, are usually dimers or trimers that are distributed randomly throughout the genomes of all eukaryotes (Tautz 1989; Quellar et al. 1993; Bruford and Wayne 1993) and have high mutation rates (Weber and May 1989). The high degree of polymorphism found in microsatellites and the ease with which these can be characterized using PCR and polyacrylamide gel electrophoresis lend them to use in genetic distance studies. To date, although steadily increasing, studies carried out to catalogue the microsatellite polymorphisms that exist among and within cattle populations remain fairly limited (MacHugh et al. 1994, 1998; Moazami-Goudarzi et al. 1997; Peelman et al. 1998; Schmid et al. 1999; Hanotte et al. 2000). The FAO and the International Livestock Research Institute (ILRI) are now engaged in a coordinated global effort to do just that. Canadienne cattle, being a unique breed and given their current struggle for survival, are also prime candidates for examination using this approach. Thus, the objective of this study was to determine the genetic distance among the Canadienne, Holstein, Jersey, and Brown Swiss cattle breeds found in Canada using 15 different, previously mapped, non sex-linked microsatellite markers located on separate chromosomes and to draw conclusions about the relationship among and within these breeds.

Materials and methods

Animals

Pedigree records provided by Canadian Livestock Records Corporation and Holstein Canada were edited before computing the additive relationship coefficients for individual animals (Cruden 1949; Emik and Terrill 1949; Van Vleck 1993). The choice of animals for sampling was based on their relationship with each other. Every effort was made to identify unique animals. Concurrently, a reasonable driving distance from the laboratory in Ottawa was also considered while selecting animals for sampling. In total, 21 Canadienne, 20 Brown Swiss, and 20 Jersey cows were sampled. The average relationship among the selected animals was 2.20% for Canadienne, 1.05% for Brown Swiss, and 2.10% for Jersey cows. The 20 Holstein bulls sampled had no common ancestor for three generations. All animals used in this study were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care.

Extraction of total genomic DNA from whole blood samples

Blood samples were collected from individual animals in 8.5-mL ACD-Vaccutainer tubes (VWR Scientific, Ville Mont-Royal, Qué.; ACD: 22.0 g trisodium citrate/L, 8.0 g citric acid/L, and 24.5 g dextrose/L) and kept at 4°C until the time of the DNA extraction. Total genomic DNA was extracted from individual blood samples using the Super Quik-Gene DNA isolation kit (Analytical Genetic Testing Center, Inc., Denver, Colo.) following the manufacturer's instructions. DNA was extracted from the blood samples within a day or two of collection.

PCR and gel electrophoresis

The samples of extracted DNA were used to perform PCRs to amplify specific microsatellite sequences. The loci studied and primers used in these reactions are shown in Table 1. Primers were end labeled using [^{32}P]ATP (Mandel Scientific Company Ltd., Guelph, Ont.) and T4 polynucleotide kinase (Promega, Madison, Wis.). Approximately 25 ng of genomic DNA per PCR was used. These samples were amplified in a reaction volume of 30 μL containing 21.7 μL of deionized, distilled water, 3.0 μL of 10× PCR buffer, 2.0 μL of 25mM $MgCl_2$, 0.8 μL of a mixture of 10 mM each dNTP, 0.8 μL of DMSO, 1.0 μL of the labeled forward primer, 0.5 μL of the reverse primer, and 0.2 uL of Ampli*Taq* DNA polymerase (Applied Biosystems Canada Inc., Mississauga, Ont.). A standard thermocycling protocol of 3 min at 94°C, followed by 30 cycles of 1 min at 94°C,

Table 1. Data on the microsatellite loci examined in the study.

Chromosome	Locus	Primers (5′→3′)	T_A (°C)	No. of alleles	Size (bp)	Reference
1	BM 864	f: TGGTAGAGCAATATGAAGGCC r: GGAAATCCAAGAAAGAGGGG	58	14	214–274	Bishop et al. 1994
2	BM 2113	f: GCTGCCTTCTACCAAATACCC r: CTTCCTGAGAGAAGCAACACC	58	11	123–143	Bishop et al. 1994
7	BM6501	f: ACTAATAAGAAATTCTGCATGTG r: TGCCACCATGACTCAGAAGTAGTTC	58	8	87–107	Stone et al. 1995
8	CSSM47	f: TCTCTGTCTCTATCACTATATGGC r: CTGGGCACCTGAAACTATCATCAT	58	8	~150	Barendse et al. 1994
10	BM 1237	f: TCATCTTGGGCATAAGACAGG r: ATTGTTCCCAGCATCTTAGAGG	58	9	187–223	Bishop et al. 1994
13	BM 720	f: ACATCTCATTCTTGTGTCATGG r: GAAATTCAGTTTAGGGTTCCCC	56	14	210–240	Bishop et al. 1994
15	ADCY2	f: AAAGTGACACAACAGCTTCTCC r: ACAAGTGAGTGCGTAACAAAGG	54	7	185–205	Bishop et al. 1994
16	BM 121	f: TGGCATTGTGAAAAGAAGTAAA r: ACTAGCACTATCTGGCAAGCA	58	14	118–160	Bishop et al. 1994
19	BP 20	f: TCTGTGGGTGAACAAGCAAG r: GGCTCCCTAAAGACCCACTC	56	8	219–233	Bishop et al. 1994
21	BM 3413	f: TCCCTGGTAACCAATGAATTC r: CAATGGATTTGACCCTCCC	58	9	170–192	Bishop et al. 1994
24	BM 226	f: ATTGCCTTGTCCGTGTATCC r: CCGGCTGAATTGCTATAAGC	58	10	128–164	Bishop et al. 1994
25	BMC8012	f: AATTCCATGCACAGAGGACC r: GATTCCAGAAAGTTCCCCCA	58	10	197–215	Bishop et al. 1994
27	BM 203	f: GGGTGTGACATTTTGTTCCC r: CTGCTCGCCACTAGTCCTTC	58	11	203–233	Bishop et al. 1994
28	BM 2515	f: GATTCCCTGACTCTCTGTCCC r: AGTATTGGCAAGTCAATGGAGG	58	5	132–148	Bishop et al. 1994
X	BM 6017	f: TCTTCTGTTTTCCTCCATCCC r: GGAAACTAGCTTATGCTGTGGG	58	10	112–139	Bishop et al. 1994

Note: f, forward; r, reverse; T_A, annealing temperature.

30 s at the annealing temperature (Table 1) and 1 min at 72°C, and ending with an extension phase of 4 min at 72°C was used. An MJ Research DNA engine was employed for all PCR runs (MJ Research, Waltham, Mass.). The amplified product was diluted with 20 µL of "stop" solution (95% v/v formamide, 20 mM EDTA, 0.05% w/v bromophenol blue, 0.05% w/v xylene cyanol FF) and stored at –20°C for a maximum of 3 days before use.

A "T ladder" reference marker for the subsequent gel electrophoresis was made using the Sequenase version 2.0 DNA Sequencing Kit (Amersham Canada Ltd., Oakville, Ont.) following the manufacturer's instructions. All amplified samples and the ^{35}S-labeled T ladder reference marker were denatured by incubation at 94°C for 5 min, and run on a 6% w/v denaturing polyacrylamide gel (thickness 0.4 mm) in 1× TBE at 50°C and 123 W on a BIO-RAD sequencing apparatus (BIO-RAD Laboratories (Canada) Ltd., Mississauga, Ont.). The gel was then transferred to Whatman 3MM paper, covered in plastic wrap, and dried for 2.5 h on a BIO-RAD gel dryer. All dried gels were exposed overnight to Kodak BIOMAX film (Kodak, Ottawa, Ont.) and developed in an Ecomat 4000 automatic developer. Allele sizes were approximated by comparison with the reference marker and each animal was scored to establish the specific alleles present at each microsatellite locus.

Analysis of the data

To facilitate the analysis, the MICROSAT 1.5b computer package (Minch 1997) was used. This program allowed for the efficient calculation of allele size variance (calculated as a simple variance) and heterozygosity (estimated directly from the observed data; expected values are estimated from Hardy–Weinberg proportions). Bootstrapping was carried out over 1000 replications in all cases. All loci were tested for divergence from Hardy–Weinberg equilibrium using the GENEPOP version 3.0 computer program (Raymond and Rousset 1995). Genetic distances were estimated among the four breeds using the allele frequency data collected by scoring the individual animals for 15 microsatellite markers. Methodologies to determine genetic distances have been described for one locus (which may be easily extended to more loci by summation and dividing by the number of alleles). In this study, three mutation-based distance measures, namely Nei's standard genetic distance (D; Nei 1972), Goldstein's genetic distance ($(\delta\mu)^2$; Goldstein et al. 1995), and Slatkin's genetic distance (R_{st}; Slatkin 1995) were used. In addition to

Table 2. Breed-specific microsatellite alleles observed in Canadienne (CN; n = 20), Brown Swiss (BS; n = 20), Jersey (JE; n = 20), and Holstein (HO; n = 20) cattle populations.

Locus	Allelic frequency (%)	Allele (bp)	Breed
BM 864	29	274	CN
		264	BS
		258	CN
		226	HO
BM 2113	15	134	JE
		132	JE
BM 6501	8	108	HO
		92	BS
CSSM 47	18	172	JE
BM 720	22	242	HO
		230	HO
		220	CN
BP 20	20	237	HO
		225	HO
		221	HO
BM 226	10	154	CN
		138	CN
BM 203	25	229	JE
		223	HO
		211	HO
BM 2515	25	134	BS
BM 6017	8	143	CN
		117	HO

the above procedures, the drift-only model-based coefficient F_{st} (Reynolds et al. 1983) was included for the purpose of comparison. Phenograms were constructed from the different mutation-based genetic distance measures using the Drawgram program of the PHYLIP 3.5c package (Felsenstein 1989).

Results

The average number of alleles per locus for all of the microsatellites was 6.3 ± 0.64 for Canadienne, 6.3 ± 0.49 for Brown Swiss, 4.9 ± 0.47 for Jersey, and 6.1 ± 0.49 for Holstein cattle. Though not significant, Jersey cattle had the lowest number of alleles. When the 15 microsatellite loci were examined for breed-specific alleles, a total of 6 alleles at BM864, BM720, BM226, and BM6017 loci were found to be specific for the Canadienne breed; 3 alleles at loci BM864, BM6501, and BM2515 for the Brown Swiss breed; 4 alleles at loci BM2113, CSSM47, and BM203 for the Jersey breed; and 10 alleles at loci BM864, BM6501, BM720, BP20, BM203, and BM6017 for the Holstein breed. The combined frequency of the breed-specific alleles found at each locus varied from 8 to 29% (Table 2).

The allelic frequencies varied among breeds at most loci. In fact, only five of the loci (BM864, BM1237, BP20, BM3413, and BM226), were found to have the same predominant allele in each of the breeds. The allele size variances among the 15 microsatellite loci ranged from 0.72 for locus BM3413 to 21.49 for locus BM1237 (Table 3). In contrast, these variances that varied among loci were similar for all four breeds of cattle (P > 0.05). The average heterozygosity

Table 3. Mean (±SE) for heterozygosity and allele size variation by breed and microsatellite loci.

Source	Heterozygosity	Allele size variance
Breed		
Canadienne	0.69	7.91
Brown Swiss	0.68	7.76
Holstein	0.69	8.08
Jersey	0.59	8.08
Locus		
BM 864	0.60	12.90
BM 2113	0.74	4.72
BM 6501	0.74	4.85
CSSM 47	0.43	8.12
BM1237	0.72	21.49
BM 720	0.70	5.79
ADCY2	0.67	4.49
BM121	0.78	21.17
BP 20	0.46	1.78
BM3413	0.45	0.72
BM 226	0.73	8.85
BMC8012	0.69	1.74
BM 203	0.77	9.77
BM 2515	0.61	2.89
BM 6017	0.83	9.32

was relatively high among the 15 microsatellite loci, ranging from 0.43 to 0.83. However, although the heterozygosity was similar in the Canadienne, Brown Swiss, and Holstein breeds, the Jersey breed tended to have a slightly lower estimate (P > 0.05).

An examination of all microsatellites across breeds revealed 13 microsatellite loci were in Hardy–Weinberg equilibrium; the exceptions were loci BM2113 and BM6501. When breeds were examined individually, there was disequilibrium at one locus (ADCY2) for Canadienne, at two loci (BM3413 and BM203) for Brown Swiss, at three loci (BM2113, BM3413, and BM6501) for Jersey, and at five loci (CSSM47, BM720, BP20, BMC8012, and BM6501) for Holstein cattle.

The genetic distances among cattle breeds were estimated using three mutation-based distance measures and a drift-only model all based on allelic frequencies of microsatellite loci (Table 4). All estimates of genetic distances were significantly different from zero. Though not significant, the genetic distance varied and Nei's estimate of standard genetic distance ranged from 0.156 to 0.427. The genetic distance between Canadienne and Holstein cattle tended to be lower (0.156) than those between Canadienne and Brown Swiss (0.243) or Jersey (0.235) cattle, whereas Brown Swiss and Jersey breeds (0.427) tended to be most distantly related. The Goldstein estimate of genetic distance ranged from 1.301 to 3.717. Again, the individual values did not differ significantly from one another. Goldstein's procedure, however, indicated that the Canadienne breed was more closely related to the Brown Swiss (3.385) and Holstein (3.136) breeds, and Brown Swiss and Holstein (1.301) cattle were not far apart. In general, Slatkin's estimate of genetic distance was similar in overall results to the Goldstein's ranging from 0.075 to 0.202. The F_{st} coefficient based on the

Table 4. Genetic distance estimates (±SE) among Canadienne, Brown Swiss, Jersey, and Holstein cattle derived using four different genetic distance measures.

(*a*) Nei's genetic distance (*D*)			
	Canadienne	Brown Swiss	Jersey
Brown Swiss	0.243 ± 0.078		
Jersey	0.235 ± 0.066	0.427 ± 0.124	
Holstein	0.156 ± 0.057	0.211 ± 0.047	0.320 ± 0.084
(*b*) Goldstein's genetic distance ($(\delta\mu)^2$)			
	Canadienne	Brown Swiss	Jersey
Brown Swiss	3.385 ± 1.204		
Jersey	3.717 ± 1.114	3.672 ± 1.492	
Holstein	3.136 ± 1.166	1.301 ± 0.443	3.088 ± 1.158
(*c*) Slatkin's genetic distance (R_{st})			
	Canadienne	Brown Swiss	Jersey
Brown Swiss	0.134 ± 0.040		
Jersey	0.202 ± 0.052	0.146 ± 0.042	
Holstein	0.142 ± 0.040	0.075 ± 0.030	0.129 ± 0.040
(*d*) Reynold's coefficient of coancestry (F_{st})			
	Canadienne	Brown Swiss	Jersey
Brown Swiss	0.121 ± 0.038		
Jersey	0.135 ± 0.026	0.190 ± 0.036	
Holstein	0.079 ± 0.021	0.095 ± 0.017	0.154 ± 0.026

drift-only model produced results that were consistent with those described earlier, and the coefficients did not vary significantly. Canadienne cattle were found to be most closely related to Holstein cattle (0.079) and most distantly related to Jersey cattle (0.135). Brown Swiss and Holstein cattle again were found to be very similar. Phenograms showing the relationships derived using the different mutation-based methods are shown in Fig. 1.

Discussion

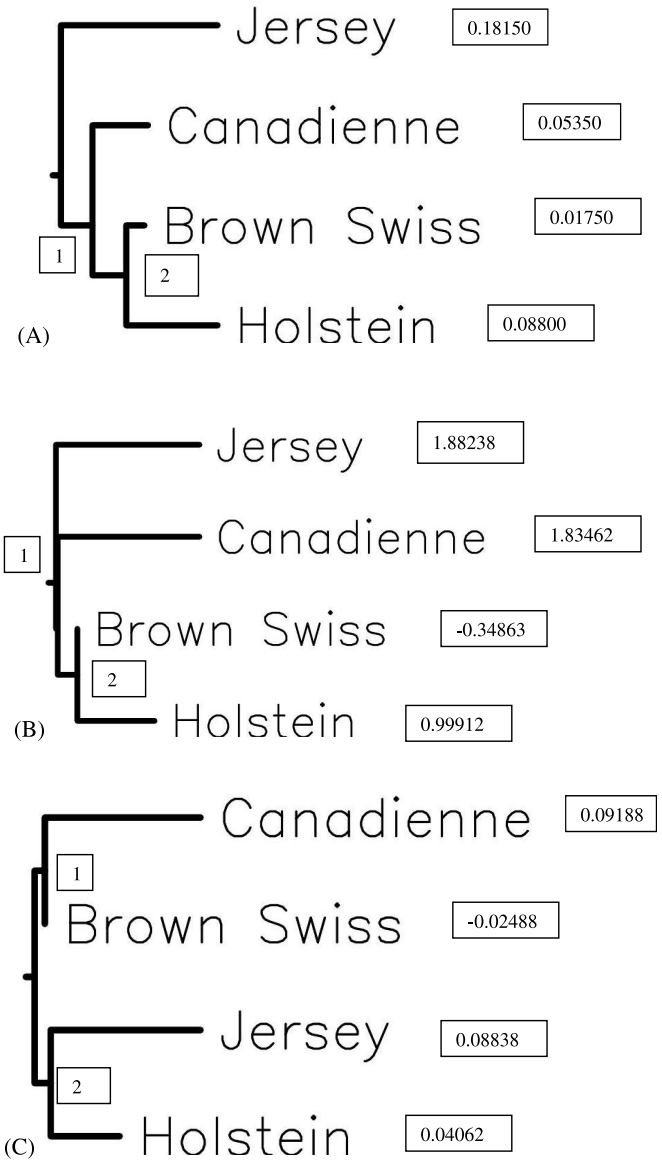
In December 1993, with the formal ratification of the Convention on Biodiversity (CBD), the issue of conservation and management of global animal genetic resources came to the forefront in many countries of the world. Governments have become more aware of the fact that valuable genetic resources are being lost at an alarming rate. In fact, estimates made by the FAO (Scherf 2000) have put the rate of loss of agriculturally useful breeds at as many as one a week. Although the actual implementation of a sustainable program for the conservation of animal genetic resources requires a wide variety of approaches and technologies, one very basic requirement is the characterization of the breeds at our disposal. Such characterizations in the past have taken the form of historical, pedigree, and production records and physical descriptions. With the growing availability of new techniques in molecular biology, it is now possible to characterize genetic resources at the DNA level.

To avoid any linkage within chromosomes, microsatellite loci on separate chromosomes, known not to be sex linked, were chosen. The use of linked loci tends to introduce covariance among loci located in the same chromosome. The magnitude is dependent on the size of the important effect of the loci involved. This tends to bias the estimate of genetic distance, increasing the variance of the genetic distance. However, the use of multiple loci adds precision to the estimate of genetic distance. All 15 microsatellite loci used were polymorphic in all cattle breeds studied.

The average number of alleles per locus of five and six in the present study was in line with the recommendation by FAO suggesting at least five different alleles per locus for estimation of genetic distance. The allele size variance was similar for all the breeds studied (Table 3), but highly variable for individual loci. Brown Swiss, Canadienne, and Holstein cattle were similar in heterozygosity. The heterozygosity estimate, ranging from 0.45 to 0.83 across loci, was high. The fact that some significant deviations were observed at specific loci is in agreement with the finding of MacHugh et al. (1994) that some degree of heterogeneity can be observed among the European cattle populations.

A population is said to be in Hardy–Weinberg equilibrium if the gene (p and q) and genotype frequencies, p^2 (AA), $2pq$ (Aa), and q^2 (aa) are constant from one generation to another. Evolutionary forces such as genetic drift, selection, mutation, and migration will disrupt Hardy–Weinberg equilibrium in a population. Genetic drift results from the variances associated with small population size, where mating of closely related individuals contributes to increased inbreeding. The selective advantage of non-neutral alleles contributes to divergence among populations. Similarly, mutation at a low frequency contributes to genetic differentiation among populations measurable over a number of generations. It is interesting to note from the results that generally Hardy–Weinberg equilibrium was observed within the populations tested. Ciampolini et al. (1995) reported that Hardy–Weinberg equilibrium was not maintained in a study of microsatellites for four Italian beef breeds. Of particular interest is the fact that, within populations, Holstein cattle showed disequilibrium at the largest number of loci. This may be a result of the heavy selection pressure and greater

Fig. 1. Phenograms based on the genetic distances measures of (A) Nei (1972), (B) Goldstein et al. (1995), and (C) Slatkin (1995). Branch lengths from the nodes are shown.



use of proven bulls for Holstein cattle. The use of artificial insemination and embryo transfer is also especially widespread in the Holstein breed compared with the other three breeds and this may, therefore, be having a more pronounced genetic influence in this population.

It is still unresolved whether allelic distributions for microsatellites most closely follow the infinite alleles model of mutation (Kimura and Crow 1964), which suggests that any new mutations that occur are always different from the existing alleles in the population, or whether they should follow the stepwise mutation model (Kimura and Ohta 1978), which maintains that alleles of the same length, but of differing descent, may be produced by mutation. The three mutation-based procedures used to calculate genetic distance differ from each other in a few fundamental ways that naturally affect the results obtained from each method. Nei's standard genetic distance is an allele frequency based procedure that assumes that the infinite alleles model holds true. Goldstein's and Slatkin's measures, on the other hand, are allele size and allele size variance based procedures for estimating genetic distance that are designed to fit the stepwise mutation model. Although R_{st} represents the fraction of the total variance of allele size that is between populations, $(\delta\mu)^2$ uses the squared mean difference between the alleles of two populations to estimate genetic distance. This latter distance has the advantage of being independent of population size. It has been shown and discussed widely in the literature (Schlötterer and Tautz 1992; Goldstein et al. 1995; Slatkin 1995) that microsatellite loci appear to adhere most closely to the stepwise mutation model, especially over relatively short time scales. According to this interpretation, distances based on Nei's procedure may, therefore, be less appropriate for microsatellites. However, because standard distance has been used widely in previous studies, it was included in the present one for the sake of consistency. Again, it should be noted that genetic distance studies have been used mainly to differentiate between species where large differences may exist. It is interesting to note that, knowing the fact that the Canadienne breed was crossbred with Brown Swiss bulls in recent years, the two allele size based procedures appear to generate results that more closely agree with breed history. Both show that the Canadienne breed tends to be most closely related to the Brown Swiss and Holstein breeds, whereas with Nei's procedure there is a tendency for the Holstein breed to be more closely related to the Canadienne breed than is the Brown Swiss breed. The apparent superiority of the procedures used by Goldstein and Slatkin over Nei in accounting for breed history tends to support the opinion outlined above that the stepwise mutation model may be more suitable in comparing breeds based on microsatellite loci. In contrast, Forbes et al. (1995), in a study involving domestic and bighorn sheep, concluded that Nei's distance, among others, was more sensitive to population history than variance-based distances like R_{st}.

As was indicated earlier, the drift-only model based on the coefficient of coancestry (F_{st}) was included in the analysis for the purpose of comparison. Because the Canadienne breed, as well as the other breeds considered, is relatively new from an evolutionary standpoint, genetic drift may be affecting the populations to the same extent as mutation. As a result, a drift-only based model may be better able to account for differences between breeds. However, as can be seen, the results, in terms of the ranking of the breeds, did not differ significantly when this method was employed.

The relatively more distant relationship that was found between the Canadienne breed and the Jersey breed, particularly with the procedures of Goldstein and Slatkin (Table 4; Fig. 1), is somewhat surprising. Historically, Canadienne cattle are believed to have originated from cattle brought by the early settlers from the Normandy and Brittany regions of France. It is widely believed that the Canadienne, Jersey, Kerry, and Guernsey cattle stem from the same general lineage in the Channel Islands. In fact, Canadienne cattle are fairly similar to Jersey cattle in overall appearance and have been referred to as "Black Jerseys" on occasion. Likewise, the fact that the Holstein breed was consistently shown to be quite closely related to the Canadienne is surprising. From a strictly phenotypic standpoint there are very few similarities

between the two breeds. Furthermore, recent historical records do not indicate any direct link between these breeds. One contributing cause to their relatively close relationship may be associated with the introduction of Brown Swiss into the Canadienne breed. As can be seen from Table 4, Brown Swiss and Holstein cattle appear to be closely related.

Finally, an examination of the variations in allele frequencies found at the different loci in the four breeds and the number of apparently breed-specific alleles raises the question as to whether or not such information could be used to characterize a breed. In fact, this has been shown to be possible by Hanotte et al. (2000) in a study involving various cattle breeds. These authors were able to use combined allele frequency differences to identify an individual's breed with relative certainty. Subsequent to the present study, FAO and EAAP have released the list of a number of microsatellites to determine genetic distance for various species. The use of high-throughput automatic sequencers will certainly increase the efficiency of studying a larger number of microsatellites. More work will be required in the future, however, before the degree of breed specificity of the various alleles in this study can be substantiated.

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References

Barendse, W., Armitage, S.M., Kossarek, L.M., Shalom, A., Kirkpatrick, B.W., Ryan, A.M., Clayton, D., Li, L., Neibergs, H.L., Zhang, N., Grosse, W.M., Weiss, J., Creighton, P., McCarthy, F., Ron, M., Teale, A.J., Fries, R., McGraw, R.A., Moore, S.S., Georges, M., Soller, M., Womack, J.E., and Hetzel, D.J.S. 1994. A genetic linkage map of the bovine genome. Nat. Genet. **6**: 227–235.

Bernier, J.-G. 1995. Canadienne bovine breed report. *In* Proceedings of the 3rd Global Conference on Conservation of Domestic Animals Genetic Resources. Rare Breeds International, Kenilworth, Warwickshire, U.K. pp. 148–156.

Bishop, M.D., Kappes, S.M., Keele, J.W., Stone, R.T., Sunden, S.L.F., Hawkins, G.A., Toldo, S.S., Fries, R., Grosz, M.D., Yoo, J., and Beattie, C.W. 1994. A genetic linkage map for cattle. Genetics, **136**: 619–639.

Bretting, P.K., and Widrlechner, M.P. 1995. Genetic markers and horticultural germplasm management. Hort. Sci. **30**: 1349–1356.

Bruford, M.W., and Wayne, R.K. 1993. Microsatellites and their application to population genetic studies. Curr. Opin. Genet. Dev. **3**: 939–943.

Ciampolini, R., Moazami-Goudarzi, K., Vaiman, D., Dillman, C., Mazzanti, E., Foulley, J.L., Leveziel, H., and Cianci, D. 1995. Individual multilocus genotypes using microsatellite polymorphisms to permit the analysis of the genetic variability within and between Italian beef cattle breeds. J. Anim. Sci. **73**: 3259–3268.

Couture, J.A. 1909. French Canadian cattle. Livre de généalogie dubétail canadien. **1**: 13–18.

Cruden, D. 1949. The computation of inbreeding coefficients for closed populations. J. Hered. **40**: 248–251.

Emik, L.O., and Terrill, C.E. 1949. Systematic procedures for calculating inbreeding coefficients. J. Hered. **40**: 51–55.

Felsenstein, J. 1989. PHYLIP — phylogeny inference package version 3.2. Cladistics, **5**: 164–166.

Forbes, H.S., Hogg, J.T., Buchanan, F.C., Crawford, A.M., and Allendorf, F.W. 1995. Microsatellite evolution in congeneric mammals: domestic and bighorn sheep. Mol. Biol. Evol. **12**: 1106–1113.

Fortin, L. de G. 1940. Histoire de la race bovine Canadienne. La Bonne Terre, Ecole Supérieure d'Agriculture, Ste-Anne de la Pocatière, Qué.

Goldstein, D.B., Ruis-Linares, A., Feldman, M., and Cavelli-Sforza, L.L. 1995. Genetic absolute dating based on microsatellites and the origin of modern humans. Proc. Natl. Acad. Sci. U.S.A. **92**: 6720–6727.

Grisdale, J.H. 1909. French Canadian cattle. Livre de généalogie dubétail canadien. **1**: 40–49.

Hanotte, O., Verjee, Y., Ochieng, J., and Rege, J.E.O. 2000. Cattle microsatellite markers for amplification of polymorphic loci in Asian bovidae. *In* Proceedings of the 4th Global Conference on the Conservation of Domestic Animal Genetic Resources, 17–21 August 1998, Kathmandu, Nepal. *Edited by* J.N.B. Shrestha. Nepal Agricultural Research Council, Lalitpur, Nepal and Rare Breeds International, Shropshire, U.K. pp. 47–49.

Hansen, C. 1997. The genetic distance among cattle breeds as related to animal genetic resource conservation. Ph. D. thesis, University of Manitoba, Winnipeg, Man.

Hansen, C., Bernier, J.-G., and Shrestha, J.N.B. 2000. Broadening of the genetic base in Canadienne cattle. *In* Proceedings of the 4th Global Conference on the Conservation of Domestic Animal Genetic Resources. Nepal Agricultural Research Council, Lalitpur, Nepal and Rare Breeds International, Shropshire, U.K. pp. 152–155.

Kimura, M., and Crow, J.F. 1964. The number of alleles that can be maintained in a finite population. Genetics, **49**: 725–738.

Kimura, M., and Ohta, T. 1978. Stepwise mutation model and distribution of allelic frequencies in a finite population. Proc. Natl. Acad. Sci. U.S.A. **75**: 2868–2872.

MacHugh, D.E., Loftus, R.T., Bradley, D.G., Sharp, P.M., and Cunningham, P. 1994. Microsatellite DNA variation within and among European cattle breeds. Proc. R. Soc. Lond. B. Biol. Sci. **256**: 25–31.

MacHugh, D.E., Loftus, R.T., Cunningham, P., and Bradley, D.G. 1998. Genetic structure of seven European cattle breeds assessed using 20 microsatellite markers. Anim. Genet. **29**: 333–340.

Minch, E. 1997. Microsat 1.5b. Stanford University, Standford, Calif.

Moazami-Goudarzi, K., Laloe, D., Furet, J.P., and Grosclaude, F. 1997. Analysis of genetic relationships between 10 cattle breeds with 17 microsatellites. Anim. Genet. **28**: 338–345.

Nei, M. 1972. Genetic distance between populations. Am. Nat. **106**: 283–292.

Peelman, L.J., Mortiaux, F., Van-Zeveren, A., Dansercoer, A., Mommens, G., Coopman, F., Bouquet, Y., Burny, A., Renaville, R., and Portetelle, D. 1998. Evaluation of the genetic variability of 23 bovine microsatellite markers in four Belgian cattle breeds. Anim. Genet. **29**: 161–167.

Quellar, D.C., Strassmann, J.E., and Hughes, C.R. 1993. Microsatellites and kinship. Trends Ecol. Evol. **8**: 285–288.

Raymond, M., and Rousset, F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. **86**: 248–249.

Reynolds, J., Weir, B.S., and Cockerham, C.C. 1983. Estimation of the coancestry coefficient: basis for a short term genetic distance. Genetics, **105**: 767–779.

Scherf, B.D. 2000. World watch list of domestic animal diversity. 3rd ed. Food and Agriculture Organization of the United Nations, Rome, Italy.

Schlötterer, C., and Tautz, D. 1992. Slippage synthesis of simple sequence DNA. Nucleic Acids Res. **20**: 211–215.

Schmid, M., Saitbekova, N., Gaillard, C., and Dolf, G. 1999. Genetic diversity in Swiss cattle breeds. J. Anim. Breed. Genet. **116**: 1–8.

Shrestha, J.N.B., and Hansen, C. 1997. Canada's animal genetic resources: cattle breeds in Canada. Agriculture and Agri-Food Canada, Res. Br. Tech. Bull. 1998-2E, Ottawa, Ont.

Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics, **139**: 457–462.

Stone, R.T., Pulido, J.C., Duyk, G.M., Kappes, S.M., Keele, J.W., and Beattie, C.W. 1995. A small-insert bovine genomic library highly enriched for microsatellite repeat sequences. Mamm. Genome, **6**: 714–724.

St-Pierre, A. 1936. Notes historiques sur la race bovine canadienne. Société des éleveur de bovins canadiens. pp. 64.

Tautz, D. 1989. Hypervariability of simple sequences as a general source of polymorphic DNA markers. Nucleic Acids Res. **17**: 6463–6471.

Van Vleck, L.D. 1993. Selection index and introduction to mixed model methods. CRC Press Inc., Boca Raton, Fla.

Weber, J.L., and May, P.E. 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am. J. Hum. Genet. **44**: 388–396.