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The diversity of bovine MHC class II *DRB3* genes in Japanese Black, Japanese Shorthorn, Jersey and Holstein cattle in Japan

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Abstract

We sequenced exon 2 of the major histocompatibility complex (MHC) class II *DRB3* gene from 471 individuals in four different Japanese populations of cattle (201 Japanese Black, 101 Holstein, 100 Japanese Shorthorn, and 69 Jersey cattle) using a new method for sequence-based typing (SBT). We identified the 34 previously reported alleles and four novel alleles. These alleles were 80.0–100.0% identical at the nucleotide level and 77.9–100.0% identical at the amino acid level to the bovine MHC *(BoLA)-DRB3* cDNA clone NR1. Among the 38 alleles, eight alleles were found in only one breed in this study. However, these alleles did not form specific clusters on a phylogenetic tree of 236-base pairs (bp) nucleotide sequences. Furthermore, these breeds exhibited similar variations with respect to average frequencies of nucleotides and amino acids, as well as synonymous and non-synonymous substitutions, in all pairwise comparisons of the alleles found in this study. By contrast, analysis of the frequencies of the various *BoLA-DRB3* alleles in each breed indicated that *DRB3*1101* was the most frequent allele in Holstein cattle (16.8%), *DRB3*4501* was the most frequent allele in Jersey cattle (18.1%), *DRB3*1201* was the most frequent allele in Japanese Shorthorn cattle (16.0%) and *DRB3*1001* was the most frequent allele in Japanese Black cattle (17.4%), indicating that the frequencies of alleles were differed in each breed. In addition, a population tree based on the frequency of *BoLA-DRB3* alleles in each breed suggested that Holstein and Japanese Black cattle were the most closely related, and that Jersey cattle were more different from both these breeds than Japanese Shorthorns.

Keywords: *BoLA-DRB3* allele; Sequence-based typing; Allele frequency; Population tree; Cattle breed; Major histocompatibility complex

1. Introduction

The major histocompatibility complex (MHC) class I and class II molecules exhibit an extraordinarily high degree of genetic polymorphism compared to many vertebrate species (Kennedy et al., 2002; Otting et al., 2000; Seddon and Ellegren, 2002; Imanishi et al., 1992; Yuhki and O'Brien, 1997). The MHC polymorphism occurs predominantly at residues involved in peptide binding (Brown et al., 1993), and there is compelling evidence that the polymorphism is maintained by some form of balanced selection (Hughes and Nei, 1989). The essential role of MHC molecules in the immunological recognition of foreign peptide antigens implies that the cause of such selection is related to the effects of MHC polymorphism on host defenses against pathogens.

The MHC variability in natural populations is of great interest to evolutionary biologists because of the typically high levels of polymorphism. Consequently, representative species of several mammalian orders, including Artiodactyla, Carnivora, Cetacea, Primates, and Rodentia have been characterized with respect to MHC allelic diversity. Some

Abbreviations: MHC, major histocompatibility complex; SBT, sequence based typing; BoLA, bovine MHC; bp, base pair(s); PCR, polymerase chain reaction; SSP, sequence specific primer; TAE, Tris acetate EDTA; HLA, human MHC; DLA, dog MHC.

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species exhibit extremely high levels of MHC polymorphism (Mikko and Andersson, 1995; Yuhki and O'Brien, 1997), whereas, others are characterized by very low levels (Ellegren et al., 1993). Moreover, even in species that are characterized by high levels of MHC polymorphism, some populations are monomorphic or oligomorphic. The trans-species polymorphism hypothesis seems to provide a universal explanation for differences in levels of MHC variability and other features of the MHC in natural populations (Klein et al., 1993). Variability within and among populations of a certain species is directly related to the interplay between effective population size, time of divergence, and the intensity of selective pressure at a particular locus. The gene frequencies of various human populations were determined for human MHC (*HLA*) genetic polymorphism (Imanishi et al., 1992) in order to assist in understanding the origins of given populations. Similarly, many studies have indicates that differences exist between breeds of cattle and dogs with regard to frequencies of MHC class II alleles. In cattle, interpretations of polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) and sequencing studies indicate that there are significant differences in allelic frequencies of *BoLA-DRB3.2* in Jersey (Gilliespie et al., 1999), Holstein (Dietz et al., 1997), Argentine creole (Giovambattista et al., 2001), Japanese Shorthorn (Takeshima et al., 2002), and Brazilian dairy Gir cattle (da Mota et al., 2002). Moreover, Kennedy et al. (2002) genotyped dog MHC (*DLA*) class II alleles of over 80 breeds in domestic dogs by polymerase chain reaction-sequence specific oligonucleotide probe (PCR-SSOP) and showed considerable variation in the allelic frequencies among breeds. Thus, the frequencies of alleles of MHC genes in different populations allow the differentiation and reconstruction of genetic distances among populations, providing a molecular basis for determination of the possible common origin of populations.

Around the second century A.D., cattle migrated from North China via the Korean peninsula to Japan. This movement of cattle was accompanied by the introduction of rice cultivation. Both genetic (Namikawa, 1980) and morphological (Ogawa et al., 1989) studies have demonstrated that native Japanese cattle belong to the species *Bos taurus* and are representative of the "Turano–Mongolia" type (Felius, 1995). By contrast, at the end of the 19th century, several European breeds of cattle were introduced into Japan for enhancement of native breeds (Obata et al., 1996). Japanese Black, which is the main breed of beef cattle in Japan, has been less influenced by European breeds than other breeds in Japan and other countries. Japanese Shorthorns are derived from crosses between Shorthorn cattle and Nanbu cattle and are raised predominantly in the northern part of Japan. Jersey cattle were first bred on a small island and this breed has existed in effective genetic isolation, because of strict breeding practices, for about 200 years (French et al., 1996).

There is one predominant class II *DRB* locus in cattle, namely, bovine MHC *(BoLA)-DRB3* (Lewin, 1996), and this locus is also the most polymorphic class II locus in cattle. Sixty-four *BoLA-DRB3.2* alleles in exon 2 (http://www.projects.roslin.ac.uk/bola/drb3pcr.html; Gilliespie et al., 1999) were distinguished by the PCR-RFLP method described by van Eijk et al. (1992). However, the utility of such typing is limited in the case of alleles that are split into several further alleles at the nucleotide level. Indeed, 89 alleles have been published in various breeds of cattle by sequencing of cloned genomic DNA, cDNA or cloned products of PCR (Ammer et al., 1992; Aida et al., 1995; Russell et al., 1997, 2000; Maillard et al., 1999, 2001; Takeshima et al., 2001, 2002). Recently, we developed a more precise method for sequence-based typing (SBT), which allows the identification of specific *BoLA-DRB3* alleles in large numbers of animals (Takeshima et al., 2001). The present study, using our SBT methods, was designed to determine the nucleotide sequences of exon 2 of the *BoLA-DRB3* alleles of a total of 471 individuals belonging to four distinct breeds of cattle, namely, Japanese Black, Japanese Shorthorn, Holstein and Jersey. The frequencies of alleles and the phylogenetic relationships among *BoLA-DRB3* alleles are discussed.

2. Materials and methods

2.1. Animals and extraction of DNA

Samples from a total of 471 individuals, obtained from the Livestock Improvement Association of Japan, were examined for the distribution of *BoLA-DRB3* alleles: 201 head of Japanese Black cattle; 100 head of Japanese Shorthorn cattle; 101 head of Holstein cattle; and 69 head of Jersey cattle. High-molecular-weight DNA was prepared from whole blood with 10% sodium dodecyl sulfate (SDS) and a mixture of phenol and chloroform (Takeshima et al., 2001).

2.2. Typing by PCR with sequence-specific primers (PCR-SSP) and nucleotide sequencing

To avoid preferential allele amplification in heterozygous individuals that would presumably occur because of mismatches in the regions of primer annealing or secondary structure, the first round of amplification by PCR with the locus-specific primers ERB3N and HL031 and subsequent amplification by PCR-SSP with distinct, group-specific 3′ primers (Sp1 through Sp8) and a locus-specific 5′ primer, DRB3B, were performed as described previously. In addition, we used the locus-specific 3′ primer DRB3ALL, which allowed amplification of the alleles in all previously identified groups (Takeshima et al., 2001).

After PCR-SSP, amplified DNAs in 5 μl of the reaction mixture were fractionated by electrophoresis on a 2% agarose/Tris acetate EDTA (TAE) gel. The gel was stained with ethidium bromide and bands of DNA were visualized under UV light.

DNA sequencing of each product of PCR-SSP was performed by the dideoxy chain termination method with a BigDye™ Terminator Cycle Sequencing Ready Reaction Kit and an automated sequencer ABI PRISM™ 310 Genetic Analyzer (Applied Biosystems, Foster City, CA) or with the CEQ™ 2000 DNA analysis system (Beckman Coulter, Fullerton, CA).

2.3. Sequence analysis

General sequence analysis was performed using the Lasergene program (DNASTAR, Madison, WI). Nucleotide sequences were compared with sequences in the GenEMBL database using the BLAST algorithm. New alleles were named by the BoLA nomenclature committee.

2.4. Statistical analysis

Allele frequencies (f) were obtained by direct counting. The observed frequencies of heterozygotes ($H^{observed}$), given in Table 2, were obtained directly by dividing the number of heterozygous individuals by the total number of individuals. The expected frequencies of heterozygote ($H^{expected}$) were those expected from the Hardy–Weinberg equilibrium, as calculated using Arlequin ver. 2.000 (Schneider et al., 2000).

2.5. Calculation of genetic distances and construction of population tree

Pairwise genetic distances were estimated on the bases of Kimura's two-parameter model. The gene tree was constructed from a distance matrix that was based on Neighbor-Joining method of Saitou and Nei (1987). To test the significance of the branches, 1000 bootstrap replicate calculations were performed. An analysis of breed diversity, based on allele frequency, was performed using the computer program gd5.exe. This program generates estimates of Nei's (1972) genetic distances and the population tree was constructed by the UPGMA method. Pairwise comparisons of nucleotide and amino acid substitutions between alleles were conducted according to the number of differences. The numbers of non-synonymous and synonymous nucleotide substitutions per site were estimated for each pair as

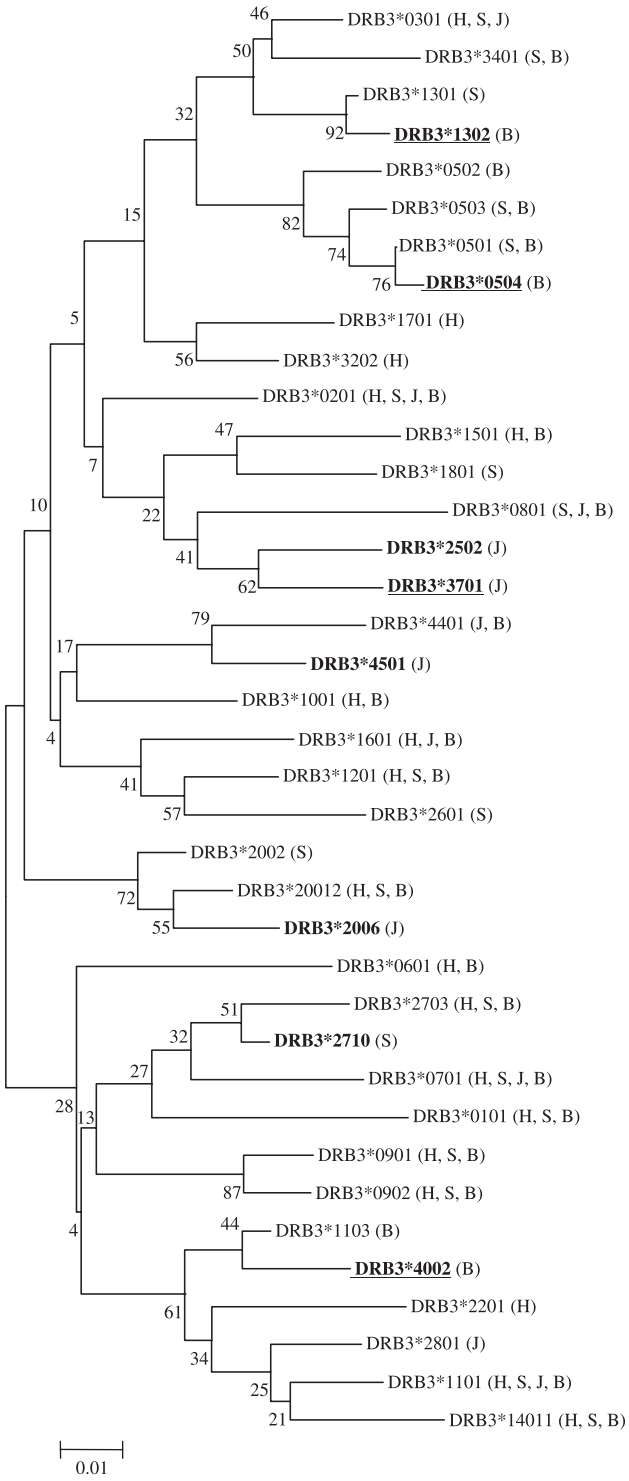


Fig. 1. Neighbor-Joining tree constructed from the 236-bp nucleotide sequences of the β1 domain coding-regions of *BoLA-DRB3* alleles derived from four distinct breeds of cattle; Holstein (H); Japanese Shorthorn (S); Japanese Black (B); and Jersey (J). The tree is based on Kimura's two-parameter distances. Numbers are bootstrap percentages that support each node. Bootstrapping was performed with 1000 replicates to assess the reliability of individual branches. Alleles that were only found in one breed in this study are shown in boldface. New alleles are underlined. Letters in parentheses indicate the breeds in which the each allele was found. The nucleotide sequences reported in this paper have been submitted to the International Nucleotide Sequence Database and have been assigned accession numbers AB033384 through AB033402, AB033404, AB033405, AB048732 through 048735, AB048814 through 048816, AB049442, AB053167, AB060152, AB060153 and Z82996.

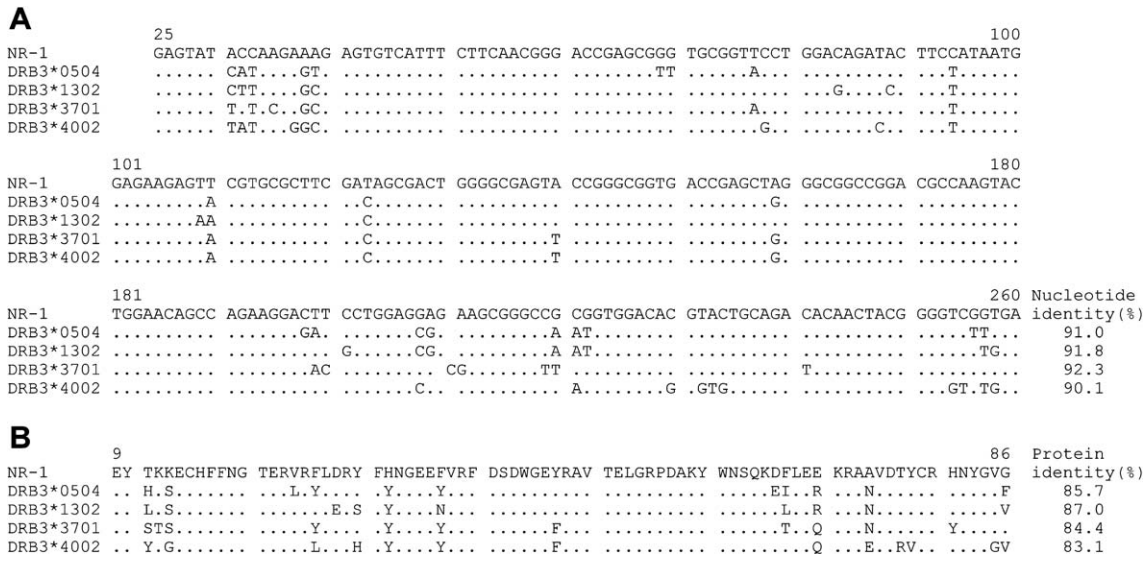


Fig. 2. Alignment of the predicted amino acid sequences of the β1 domains encoded by four new *BoLA-DRB3* alleles (accession numbers are AB053167 for *BoLA-DRB3*0504*, AB033396 for *BoLA-DRB3*1302*, Z82996 for *BoLA-DRB3*3701* and AB033404 for *BoLA-DRB3*4002*) derived from 471 animals that belonged to four distinct cattle breeds, Holstein, Japanese Shorthorn, Japanese Black and Jersey. The numbering refers to positions of amino acids in the mature protein. Amino acid residues identical to those encoded by the *BoLA-DRB3* cDNA clone NR-1 are indicated by dots (Aida et al., 1995). Homology scores also refer to this cDNA clone.

described by Nei and Gojobori (1986) using the Jukes–Cantor's formula. Analyses of phylogeny and molecular evolution were performed using MEGA version 2.1 (Kumar et al., 2001).

3. Results and discussion

3.1. Identification and characterization of BoLA-DRB3 alleles obtained from four distinct breeds of cattle

The genotypes of 471 individuals from four different Japanese populations of cattle were determined for exon 2 of the *BoLA-DRB3* allele by PCR-SBT typing (Fig. 1). The 34 previously reported alleles and four novel alleles were identified: 20 previously reported alleles were obtained from 100 Japanese Shorthorns; 14, including one new allele, from 69 Jersey cattle; 18 from 101 Holsteins; and 24, including three new alleles, from 201 Japanese Blacks.

These 38 *DRB3* alleles were 80.0–100.0% identical at the nucleotide level and 77.9–100% identical at the amino acid level to the *BoLA-DRB3* cDNA clone NR1 (Aida et al., 1995). Nucleotide sequences and deduced amino acid sequences of four new alleles are shown in Fig. 2. These four new alleles were assigned allele names by the BoLA nomenclature committee (http://www.projects.roslin.ac.uk/bola/bolahome.html). One new allele was designated *DRB3*1302* and differed from *DRB3*1301* at positions 30 and 37. Another new allele was designated *DRB3*0504* and differed from *BoLA-DRB3*0501* at position 86. The third

Table 1
Frequencies of amino acid and nucleotide substitutions in pairwise comparisons of *BoLA-DRB3* alleles[a]

Comparison[b]	Amino acid substitutions		Nucleotide substitutions		Non-synonymous substitutions[c]		Synonymous substitutions[c]	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
HO vs. HO (N=153)	0.18	0.04–0.27	0.08	0.02–0.13	0.11	0.04–0.17	0.04	0–0.12
JS vs. JS (N=190)	0.17	0.03–0.26	0.09	0.05–0.18	0.11	0.02–0.17	0.05	0–0.12
JE vs. JE (N=91)	0.18	0.06–0.24	0.08	0.03–0.12	0.11	0.05–0.16	0.05	0–0.09
JB vs. JB (N=276)	0.18	0.01–0.27	0.09	0.01–0.13	0.11	0.01–0.19	0.05	0–0.12
BoLA-DRB3 vs. *-DRB3* (N=4371)	0.17	0.00–0.29	0.08	0.00–0.14	0.11	0.00–0.20	0.04	0–0.15
Bovine TNF α vs. TNF α (N=21)	0.011	0–0.024	0.007	0.002–0.014	0.005	0–0.011	0.13	0–0.033

[a] The analysis involved codons 9 through 86 of β1 domain.
[b] N, Number of pairwise comparisons of *BoLA-DRB3* alleles from four distinct breeds of cattle, namely, Holstein (HO); Japanese Shorthorn (JS); Jersey (JE); and Japanese Black (JB); four new alleles found in this study and 89 *BoLA-DRB3* alleles had been previously published and 236 *HLA-DRB1* alleles (http://www.authonynolan.org.uk/HIG/index.html).
[c] Rates of non-synonymous and synonymous substitutions were calculated by method of Nei and Gojobori (1986).

new allele, *DRB3*4002*, differed from *DRB3*4001* at positions 12, 26, 30 and 38. The remaining new allele, *DRB3*3701*, differed from *DRB3*2501* at positions 34, 39, 57, 59 and 60.

3.2. A Neighbor-Joining tree of BoLA-DRB3 alleles obtained from the four distinct breeds of cattle

We generated a Neighbor-Joining tree using the 236-base pairs (bp) nucleotide sequences of the various β1 domains found in the four different breeds (Fig. 1). According to this tree, there were no significant differences among the alleles of all four breeds, including the four new alleles and the eight alleles that were not found in other breeds. The low bootstrap values indicated that there were no significant branches on this tree. Thus, a Neighbor-Joining tree indicates that *BoLA-DRB3* sequences isolated from one breed were shared by other breeds.

3.3. Frequencies of amino acid and nucleotide substitutions for all pairwise comparisons of BoLA-DRB3 alleles obtained from the four distinct breeds of cattle

We examined the evolution of polymorphism of the *BoLA-DRB3* gene by comparing the amino acid and nucleotide substitutions for all pairs of alleles in the four different breeds, namely, the 20 alleles in Japanese Shorthorns, the 14 alleles in Jersey cattle, the 18 alleles in Holsteins and the 24 alleles in Japanese Blacks (Table 1). The cattle populations each showed similar mean numbers of amino acid substitutions per site (average 0.17–0.18; range 0.01–0.27). Likewise, all pairwise comparisons within the 4 new alleles found in this study and the 89 *DRB3* alleles previously published averaged 0.17 (range 0.00–0.29). This result was similar to the mean of amino acid substitutions in pairwise comparisons of the 14 *BoLA-DRB3* alleles analyzed by Sigurdardottir et al. (1991). In such a comparison of polymorphic positions exclusively, there were no significant differences in terms of the mean and the range for the amino acid substitutions in the four different breeds.

Next, we calculated rates of synonymous and non-synonymous nucleotide substitution rates in all pairwise comparisons of all alleles found in the four different breeds (Table 1). We also calculated these values for the gene for bovine TNF-α as a control. There were no significant differences in terms of the mean and ranges for silent substitutions in the four different breeds. Moreover, values for non-synonymous substitutions were greater than those for synonymous substitutions in *BoLA-DRB3*, suggesting that there is selection for genetic diversity in *BoLA-DRB3* exon 2. These findings showed the same tendency as previous results by Sigurdardottir et al. (1991). By contrast, in the gene for TNF-α, values for synonymous substitutions were greater than those for non-synonymous substitutions.

Thus, all pairwise comparisons of the alleles found in this study indicate that there is no significant difference in the levels of polymorphism of *BoLA-DRB3* among the four different breeds. Therefore, these results may suggest that some alleles have been maintained intact since the divergence of different breeds. Indeed, the phylogenetic tree constructed from nucleotide sequences of *DRB3* shows that there is no typical clade for the four different breeds (Fig. 1).

3.4. Distribution of BoLA-DRB3 alleles in the four breeds of cattle

To differentiate between the allelic variations in the four distinct breeds, we determined the frequencies of *BoLA-*

Table 2
Frequencies of *BoLA-DRB3* alleles and heterozygosities (*H*) for the cattle breeds studied

DRB3 allele	Holstein[a] (*N* = 101) (allele no. 202)	Japanese Shorthorn[a] (*N* = 100) (allele no. 200)	Jersey[a] (*N* = 69) (allele no. 138)	Japanese Black[a] (*N* = 201) (allele no. 402)
0101	11.4	7.5	–	3.5
0201	5.5	7.5	16.7	4.5
0301	0.5	14.5	2.2	–
0501	–	6.5	–	0.5
0502	–	–	–	3.0
0503	–	0.5	–	2.5
0504	–	–	–	0.5
0601	4.5	–	–	–
0701	1.0	0.5	6.5	4.7
0801	–	12.0	5.1	1.0
0901	1.0	1.0	–	1.7
0902	5.9	4.5	–	7.2
1001	5.9	–	–	**17.4**[b]
1101	**16.8**[b]	8.0	2.2	11.0
1103	–	–	–	1.2
1201	14.4	**16.0**[b]	0.7	8.5
1301	–	1.0	–	–
1302	–	–	–	5.0
14011	5.0	5.0	–	2.0
1501	13.4	–	–	7.7
1601	3.5	–	2.9	12.9
1701	1.0	–	–	–
1801	–	3.5	–	–
20012	1.0	3.5	–	1.2
2002	–	3.5	–	–
2006	–	–	14.5	–
2201	0.5	–	–	–
2502	–	–	11.6	–
2601	–	0.5	–	–
2703	8.4	1.0	0.7	1.7
2710	–	0.5	–	–
2801	–	–	1.4	–
3202	0.5	–	–	–
3401	–	3.0	–	0.2
3701	–	–	10.1	–
4002	–	–	–	0.8
4401	–	–	7.2	1.2
4501	–	–	**18.1**[b]	–
$H^{observed}$	92.1	92.0	91.3	90.5
$H^{expected}$	90.1 ± 0.7	91.2 ± 0.7	88.7 ± 1.0	91.4 ± 0.5

[a] *N*, number of typed unrelated individuals. *H*, heterozygosity rate.
[b] The most frequent alleles in each breed are given in boldface.

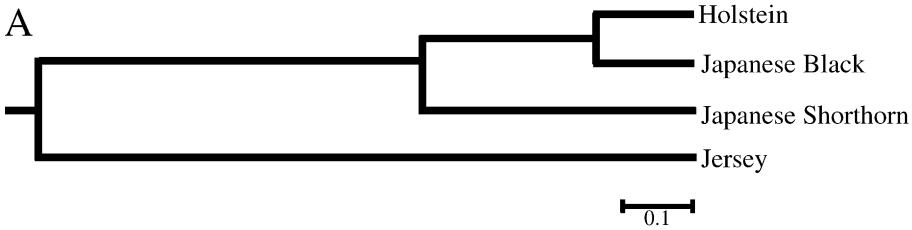
DRB3 alleles in each breed and compared them with those in other populations (Table 2). All breeds examined exhibited extremely high diversity with respect to *DRB3*, with heterozygosity between 90.5% and 94.1%, and these values were close to the expected heterozygosity of 88.7–91.4%. The *DRB3*1101* allele was the most frequent in Holsteins (16.8%) and was detected at moderate frequencies in Japanese Shorthorns (8.0%) and Japanese Blacks (11.0%). Among 24 alleles, *DRB3*1001* was the most frequent allele in Japanese Blacks (17.4%); it was infrequent in Holsteins (5.9%) and absent in Jersey cattle and Japanese Shorthorns. In addition, three alleles (*DRB3*0504*, **1302* and **4002*) were found only in Japanese Blacks, were also new alleles. In the 100 Japanese Shorthorns examined, *DRB3*1201* and *DRB3*0301* were the most common and second most common alleles with frequencies of 16.0% and 14.5%, respectively. Within the 69 Jersey cattle, the *DRB3*4501* and *DRB3*0201* alleles were the most common, with frequencies of 18.1% and 16.7%, respectively, and *DRB*4501* was not found at all in the other breeds. Similarly, three alleles (*DRB3*2006* and **2502*) were also detected only in Jersey cattle, as indicated in Fig. 1. In addition, *DRB3*3701* was found only in Jersey cattle, was also new alleles (Fig. 1).

The frequency of alleles we determined in this study generally agrees with other published research. The majority of the more abundantly (with gene frequencies higher than 5%) detected alleles in Holsteins (alleles 0101, 0201, 0902, 1001, 1101, 1201, 14011, 1501, 2703) and in Jerseys (0201, 0701, 0801, 2006, 2502, 3701, 4401, 4501) in this study are in concordance with the data previously published in other population of these breeds (e.g. Dietz et al., 1997; Sharif et al., 1998; Gilliespie et al., 1999). However, similar small differences in gene frequencies were observed among the Japanese local populations and other populations of Holsteins and Jerseys.

Our investigation clearly suggests that the remarkably dissimilar patterns of distribution of *BoLA-DRB3* alleles in the different breeds might be the result of differential selection after the separation of the major population groups of cattle. Moreover, it appears that *BoLA-DRB3* sequences isolated from one breed are shared by other breeds, suggesting that these allelic lineages might be derived from common ancestral alleles that existed prior to the divergence of these breeds.

Comparison of the frequencies of *BoLA-DRB3* alleles in breeds shows that four different breeds exhibit similarly high levels of heterozygosities. These levels are similar to those reported for Holstein and Argentinean Creole cattle (Giovambattista et al., 2001). Moreover, all pairwise comparisons of the *BoLA-DRB3* alleles suggest that there is no significant difference in the levels of polymorphism of *BoLA-DRB3* alleles among the four different breeds and values for non-synonymous substitutions were greater than those for synonymous substitutions in *BoLA-DRB3* in all four different breeds. These results provide evidence for the existence of over-dominant selection in the evolution of *BoLA-DRB3*, as in the case of the *HLA-DRB1* (Hughes and Nei, 1989).

The degrees of genetic variation at the *DRB1* loci in human and dog were measured by their heterozygosity. It appeared that there are significant differences in heterozygosities at the *HLA-DRB1* among various human populations, although values of heterozygosities of these populations tend to be similar to those of cattle (Mizuki et al., 1997). By contrast, the level of heterozygosities at *DLA-DRB1* was very low: 59.5%, and lower than those of cattle (Kennedy et al., 2002).



B

	Holstein	Jersey	Japanese Black	Japanese Shorthorn
Holstein	-			
Jersey	1.8507	-		
Japanese Black	0.2802	1.8351	-	
Japanese Shorthorn	0.5027	1.3253	0.8926	-

Fig. 3. Rooted UPGMA tree (A) constructed from Nei's genetic distances (B), which were based on the allele frequencies of alleles of the *BoLA-DRB3* gene in the four populations studied.

3.5. Population tree based on frequencies of BoLA-DRB3 alleles

Putative evolutionary relationships among different populations can be determined from the genetic distances derived from frequencies of alleles (Mizuki et al., 1997). We constructed a population tree that was based on the frequencies of *BoLA-DRB3* alleles using the UPGMA method (Fig. 3A). The smallest genetic distances for *DRB3* alleles were those between Holsteins and Japanese Blacks (0.2802) (Fig. 3B). The genetic distance between Jersey cattle and the other three breeds was large and Jerseys were clustered on a different branch from the other three breeds. Collectively, the results suggest that the Holstein and Japanese Black breeds were the closest to each other, with Jersey being farther from these two breeds than Japanese Shorthorn.

The evolution of cattle breeds has been described on the basis of variations in mitochondrial DNA using the genetic distance based on nucleotide substitution to construct the evolutionary tree. Although such analysis showed that breeds of *B. taurus* and *Bos indicus* can be clearly differentiated (Loftus et al., 1994), differences in the evolution of the various breeds were less clear. Allelic distributions of the microsatellite markers also have been analyzed with the construction of a population tree (MacHugh et al., 1997). On this tree, Simmental and Holstein were closest, and Jersey was farther from these breeds than Hereford. In the present study, we demonstrated the genetic contribution of four major breeds in Japan by constructing a population tree of four cattle breeds using their allele frequency distributions. Ours is the first tree to use the frequency of *BoLA* alleles. The introduction of foreign breeds of cattle (Brown Swiss, Shorthorn, Devon, Ayrshire, Simmental and Holstein) for crossbreeding with native cattle in Japan was encouraged by the Japanese government after the Meiji restoration in 1867. Moreover, part of the Holstein breed in Japan are crosses between Japanese native cattle and Holstein breed cattle. Our tree suggests that the Japanese Black has a great genetic contribution from Holstein cattle which breed was used to upgrade the native Japanese cattle. By contrast, although the Jersey breed was also the European breed, its genetic contribution was very limited with respect to the Japanese Black and Japanese Shorthorn breeds. It is suggested that the Japanese breeds were more similar to each other than the diversity of European breeds with respect to MHC distribution. Our present finding that Holstein and Japanese Black breeds may be more closely related to each other than to Jerseys supports the results of microsatellite DNA studies by MacHugh et al. (1997).

Cattle are grouped into two species, *B. taurus* and *B. indicus,* and *BoLA-DRB3* sequences are shared by the two groups. Japanese Black, Holstein, Japanese Shorthorn, and Jersey cattle analyzed in this study belong to *B. taurus.* Other studies describe that *B. indicus* has a quite different frequency compared with *B. taurus* and have a lot of alleles that are not found in *B. taurus* (Mikko and Andersson, 1995; Maillard et al., 1999; da Mota et al., 2002). These results indicate that many more alleles remain to be discovered. Therefore, further sequencing data from *B. indicus* are needed to characterize the variability of bovine MHC.

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References

Aida, Y., Niimi, M., Asahina, M., Okada, K., Nakai, Y., Ogimoto, K., 1995. Identification of a new bovine MHC class II DRB allele by nucleotide sequencing and an analysis of phylogenetic relationships. Biochem. Biophys. Res. Commun. 209, 981–988.

Ammer, H., Schwaiger, F.-W., Kammerbauer, C., Gomolka, M., Arriens, A., Lazary, S., Epplen, J.T., 1992. Exonic polymorphism vs. intronic simple repeat hypervariability in MHC-DRB genes. Immunogenetics 35, 332–340.

Brown, J.H., Jardetzky, T.S., Gorga, J.C., Stern, L.J., Urban, R.G., Strominger, J.L., Wiley, D.C., 1993. Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. Nature 364, 33–39.

da Mota, A.F., Gabriel, J.E., Martinez, M.L., Coutinho, L.L., 2002. Distribution of bovine lymphocyte antigen (BoLA-DRB3) alleles in Brazilian dairy Gir cattle (*Bos indicus*). Eur. J. Immunogenet. 29, 223–227.

Dietz, A.B., Detilleux, J.C., Freeman, A.E., Kelley, D.H., Stabel, J.R., Kehrli Jr., M.E., 1997. Genetic association of bovine lymphocyte antigen DRB3 alleles with immunological traits of Holstein cattle. J. Dairy Sci. 80, 400–405.

Ellegren, H., Davies, C.J., Andersson, L., 1993. Strong association between polymorphisms in an intronic microsatellite and in the coding sequence of the BoLA-DRB3 gene: implications for microsatellite stability and PCR-based DRB3 typing. Anim. Genet. 24, 269–275.

Felius, M., 1995. Cattle Breeds—An Encyclopedia. Misset, Doetinchem, The Netherlands.

French, M.H., Johansson, I., Joshi, N.R., McLaughlin, E.A., 1996. European Breeds of Cattle. Food and Agricultural Organization of United Nations, Rome.

Gilliespie, B.E., Jayarao, B.M., Dowlen, H.H., Oliver, S.P., 1999. Analysis and frequency of bovine lymphocyte antigen DRB3.2 alleles in Jersey cows. J. Dairy Sci. 82, 2049–2053.

Giovambattista, G., Ripoli, M.V., Peral-Garcia, P., Bouzat, J.L., 2001. Indigenous domestic breeds as reservoirs of genetic diversity: the Argentinean Creole cattle. Anim. Genet. 32, 240–247.

Hughes, A.L., Nei, M., 1989. Nucleotide substitution at major histocompatibility complex class II loci: evidence for overdominant selection. Proc. Natl. Acad. Sci. U. S. A. 86, 958–962.

Imanishi, T., Akaza, T., Kimura, A., Tokunaga, K., Gojobori, T., 1992. Allele and haplotype frequencies for HLA and complement loci in various ethnic groups. In: Tsuji, K., Aizawa, M., Sasazuki, T. (Eds.), HLA 1991, vol. 1. Oxford Univ. Press, New York, pp. 1065–1220.

Kennedy, L.J., Barnes, A., Happ, G.M., Quinnell, R.J., Bennett, D., Angles, J.M., Day, M.J., Carmichael, N., Innes, J.F., Isherwood, D., Carter, S.D., Thomson, W., Ollier, W.E.R., 2002. Extensive interbreed, but minimal intrabreed, variation of DLA class II alleles and haplotypes in dogs. Tissue Antigens 59, 194–204.

Klein, J., Satta, Y., O'HUigin, C., Takahata, N., 1993. The molecular

descent of the major histocompatibility complex. Annu. Rev. Immunol. 11, 269–295.

Kumar, S., Tamura, K., Jakobsen, I.B., Nei, M., 2001. MEGA2: molecular evolutionary genetics analysis software. Bioinformatics 17, 1244–1245.

Lewin, H.A., 1996. Genetic organization, polymorphism, and function of the bovine major histocompatibility complex. In: Schook, L.B., Lamont, S.J. (Eds.), The Major Histocompatibility Complex Region of Domestic Animal Species. CRC Press, Boca Raton, FL, pp. 65–98.

Loftus, R.T., MacHugh, D.E., Bradley, D.G., Sharp, P.M., Cunningham, P., 1994. Evidence for two independent domestications of cattle. Proc. Natl. Acad. Sci. U. S. A. 91, 2757–2761.

MacHugh, D.E., Shriver, M.D., Loftus, R.T., Cunningham, P., Bradley, D.G., 1997. Microsatellite DNA variation and the evolution, domestication and phylogeography of taurine and zebu cattle (*Bos taurus* and *Bos indicus*). Genetics 146, 1071–1086.

Maillard, J.C., Renard, C., Chardon, P., Chantal, I., Bensaid, A., 1999. Characterization of 18 new BoLA-DRB3 alleles. Anim. Genet. 30, 200–203.

Maillard, J.C., Chantal, I., Berthier, D., 2001. Sequencing of four new BoLA-DRB3 and six new BoLA-DQB alleles. Anim. Genet. 32, 44–46.

Mikko, S., Andersson, L., 1995. Extensive MHC class II DRB3 diversity in African and European cattle. Immunogenetics 42, 408–413.

Mizuki, M., Ohno, S., Ando, H., Sato, T., Imanishi, T., Gojobori, T., Ishihara, M., Ota, M., Geng, Z., Geng, L., Li, G., Kimura, M., Inoko, H., 1997. Major histocompatibility complex class II alleles in Kazak and Han populations in the Silk Route of northwestern China. Tissue Antigens 50, 527–534.

Namikawa, T., 1980. Genetical aspect of domestication and phylogeny in the cattle. Jpn. J. Zoothechnol. Sci. 49, 817–827.

Nei, M., 1972. Genetic distance between populations. Am. Natur. 105, 385–398.

Nei, M., Gojobori, T., 1986. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. Mol. Biol. Evol. 3, 418–426.

Obata, T., Saitoh, M., Takeda, H., 1996. Conservation and utilization of beef cattle genetic resources in Japan. FAO Anim. Genet. Resour. Information 18, 65–78.

Ogawa, Y., Daigo, M., Amasaki, H., 1989. Craniometrical estimation of the native Japanese Mishima cattle. Anat. Anz. Jena 168, 197–203.

Otting, N., de Groot, N.G., Noort, M.C., Doxiadis, G.G.M., Bontrop, R.E., 2000. Allelic diversity of Mhc-DRB alleles in rhesus macaques. Tissue Antigens 56, 58–68.

Russell, G.C., Davies, C.J., Andersson, L., Ellis, S.A., Hensen, E.J., Lewin, H.A., Mikko, S., Muggli-Cockett, N.E., van der Poel, J.J., 1997. BoLA class II nucleotide sequences, 1996: report of the ISAG BoLA Nomenclature Committee. Anim. Genet. 28, 169–180.

Russell, G.C., Fraser, D.C., Craigmile, S., Oliver, R.A., Dutia, B.M., Glass, E.J., 2000. Sequence and transfection of BoLA-DRB3 cDNAs. Anim. Genet. 31, 219–222.

Saitou, N., Nei, M., 1987. The Neighbor-Joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.

Schneider, S., Roessli, D., Excoffier, L., 2000. Arlequin: a software for population genetics data analysis. Ver 2.000. Genetics and Biometry Lab., Dept. of Anthropology. University of Geneva.

Seddon, J.M., Ellegren, H., 2002. MHC class II genes in European wolves: a comparison with dogs. Immunogenetics 54, 490–500.

Sharif, S., Mallard, B.A., Wilkie, B.N., Sargeant, J.M., Scott, H.M., Dekkers, J.C., Leslie, K.E., 1998. Associations of the bovine major histocompatibility complex DRB3 (BoLA-DRB3) alleles with occurrence of disease and milk somatic cell score in Canadian dairy cattle. Anim. Genet. 29, 185–193.

Sigurdardottir, S., Borsch, C., Gustafsson, K., Andersson, L., 1991. Cloning and sequence analysis of 14 DRB alleles of the bovine major histocompatibility complex by using the polymerase chain reaction. Anim. Genet. 22, 199–209.

Takeshima, S., Ikegami, M., Morita, M., Nakai, Y., Aida, Y., 2001. Identification of new cattle BoLA-DRB3 alleles by sequence-based typing. Immunogenetics 53, 74–81.

Takeshima, S., Nakai, Y., Ohta, M., Aida, Y., 2002. Characterization of DRB3 alleles in the MHC of Japanese shorthorn cattle by polymerase chain reaction-sequence-based typing. J. Dairy Sci. 85, 1630–1632.

van Eijk, M.J., Stewart-Haynes, J.A., Lewin, H.A., 1992. Extensive polymorphism of the BoLA-DRB3 gene distinguished by PCR-RFLP. Anim. Genet. 23, 483–496.

Yuhki, N., O'Brien, S.J., 1997. Nature and origin of polymorphism in feline MHC class II DRA and DRB genes. J. Immunol. 158, 2822–2833.