

Gene Frequency Distribution of the *BoLA-DRB3* Locus in Saavedreño Creole Dairy Cattle

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*The objective of this study is to describe the gene frequency distribution of the bovine lymphocyte antigen (BoLA)-DRB3 locus in Saavedreño Creole dairy cattle and to compare it with previously reported patterns in other cattle breeds. One hundred and twenty-five Saavedreño Creole dairy cattle were genotyped for the BoLA-DRB3.2 allele by polymerase chain reaction and restriction fragment length polymorphism. Twenty-two out of 53 previously identified BoLA-DRB3.2 alleles were detected, with gene frequencies ranging from 0.4 to 16.8%. Seventy percent of the variation corresponded to the seven most frequent alleles (BoLA-DRB3.2*7, *8, *11, *16, *27, *36, and *37). The studied population exhibits a high degree of expected heterozygosity ($h_e = 0.919$). The F_{IS} index did not show significant deviation from Hardy-Weinberg equilibrium. However, the neutrality test showed an even gene frequency distribution. This result could be better explained assuming balancing selection instead of neutral or positive selection for one or a few alleles. In conclusion, the results of this study demonstrated that BoLA-DRB3.2 is a highly polymorphic locus in Saavedreño Creole dairy cattle, with significant variation in allele frequency among cattle breeds.*

KEY WORDS: *BoLA-DRB3.2*; Creole cattle; PCR-RFLP; genetic diversity.

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INTRODUCTION

The bovine lymphocyte antigen (*BoLA*) system constitutes the major histocompatibility complex (*MHC*) of cattle. The genes located in the *MHC* class II region encode glycoproteins that are composed of α - and β -chains and expressed on the surface of antigen-presenting cells (Klein, 1986). These molecules bind processed peptides in an endocytic compartment before being transported to the cell surface where the class II molecules-foreign antigens are presented specifically to stimulate helper T-lymphocytes. This process initiates the immune response. Therefore, *MHC* loci play an essential role in the immune response to foreign agents.

The *MHC* loci show a high degree of genetic polymorphism, presenting an excess of nonsynonymous substitutions at peptide binding sites. In addition, the allele frequency distribution is significantly more balanced than that expected for selectively neutral alleles because of the effect of balancing selection. Moreover, an excess of heterozygous individuals occurred, potentially as a consequence of overdominant selection, and the persistence of allelic lineages is longer than that expected for selectively neutral alleles (Hedrick, 1994).

In cattle, the *BoLA-DR* region is composed of one *DRA* locus and at least three *DRB* loci, *DRB1*, 2, and 3. The *BoLA-DRB3* locus is expressed at high levels while the *DRB1* is expressed at a low level. *DRB2* is a pseudogene (Burke *et al.*, 1991). Furthermore, the *BoLA-DRB3* gene exhibits a high degree of genetic polymorphism that occurs predominantly at the peptide binding sites (codified by exon 2) (Ellis and Ballingal, 1999).

The extensive structural polymorphism of the class II molecules is considered a potential cause of differences among individuals in the immune response to infectious agents. Furthermore, analysis of *DRB3.2* polymorphism is of interest because numerous associations between *BoLA* genes and susceptibility/resistance to infectious diseases like mastitis due to *Staphylococcus aureus*, bovine leukosis virus lymphocytosis, and production traits (i.e., fertility) have been found (Dietz, Dietz *et al.*, 1997a,b; Lewin, 1994; Schmutz *et al.*, 1992; Sharif *et al.*, 1998).

The objective of this study is to describe the gene frequency distribution of the *BoLA-DRB3.2* locus in Saavedreño Creole dairy cattle and to compare it with previously reported allele frequencies in other cattle breeds. Information about bovine *MHC* polymorphisms is important in the dairy industry since *MHC* contributes substantially to fitness and resistance/susceptibility to disease. Therefore, understanding of *MHC* diversity could be useful to increase dairy production in tropical regions where European dairy breeds perform poorly.

MATERIALS AND METHODS

Characteristics of the Studied Population

The Saavedra Experimental Station (Santa Cruz de la Sierra, Bolivia) was established in 1970 with the aim of milk production improvement through selection

of different Creole cattle breeds well adapted to humid tropical environments. The founder group was made up of bovines belonging to different populations of Creole cattle, commonly called Yacumeño Creole cattle (El Beni, Bolivia), Chaqueño Creole cattle (Bolivian Chaco), dairy Caracu (Minas Gerais, Brazil), Nicaraguan dairy cattle, and Cuban dairy Creole cattle. All animals were introduced at the same time, and when the first generation was achieved, the Saavedreño population was isolated and bred as an outbreed herd without use of formal crosses. Currently, the population includes about 300 adult animals (<http://www.fao.org>).

The Saavedreño Creole is a humpless breed, with uniform coat color and long horns (characteristic of American Creole cattle breeds). Males and females have an average weight of 730 and 426 kg, respectively. This breed shows a high degree of fertility and a trend to disease resistance, showing lack of mastitis and foot problems. In addition, the Saavedreño dams can produce more than 1000 L of milk in the first lactation in humid tropical environments. The animals are kept outdoors (without housing) all year and maintained under extensive stationary pastoral conditions with only a mineral supplement.

DNA Isolation

One hundred and twenty-five cattle of the Saavedreño Creole breed from the Saavedra Experimental Station were genotyped for the *BoLA-DRB3.2* locus. These animals represented all original lineages and were selected on the basis of available pedigree data. Approximately 10 mL of blood was collected from each animal via the jugular vein. The whole blood was preserved in acid citrate dextrose solution and stored at -70°C . Genomic DNA was isolated from peripheral blood lymphocytes by the DNAzol method (Invitrogen, Carlsbad, CA).

BoLA-DRB3.2 Gene Amplification

The second exon of the locus *BoLA-DRB3* was genotyped through a heminested PCR-RFLP method as previously described by van Eijk *et al.* (1992). The first PCR round was carried out in a final volume of 25 μL containing: 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM MgCl_2 , 0.1% Triton X-100, 100 mM of each dNTP, 1 μL of Taq polymerase (Invitrogen, Carlsbad, CA), 0.5 mM of each HL030 and HL031 primers, and 100 ng of DNA. The cycling conditions were as follows: an initial denaturation step of 4 min at 94°C followed by 9 cycles of 1 min at 94°C , 2 min at 60°C , and 1 min at 72°C . The last polymerization step was extended for 10 min at 72°C .

Two microliters of the amplicon from the first reaction was transferred to a new tube with 48 μL of PCR buffer containing primers HL030 and HL032, and 2 μL of Taq polymerase at the same concentrations as described above. The cycling conditions for the second round of PCR were as follows: 20 cycles of 1 min at

94°C and 90 s at 65°C, as the annealing-extension step. The last polymerization step was extended for 4 min at 72°C.

PCR-RFLP Analysis of the *BoLA-DRB3* Second Exon

The polymorphism of the second exon of the *BoLA-DRB3* gene was analyzed by restriction fragment length polymorphism (RFLP). Twelve microliters from the second PCR round was digested with 2.5 μ L of *Hae*III (Invitrogen, Carlsbad, CA), *Bst*YI (New England BioLab, Beverly, MA), and *Rsa*I (Invitrogen, Carlsbad, CA) restriction enzymes and in separated reactions with a final volume of 17 μ L. As a size marker, a *Msp*I digest pBR322 was used (New England BioLab, Beverly, MA).

Polyacrylamide Gel Electrophoresis

The restriction fragments were electrophoresed in 6%/1X TBE (19:1) acrylamide-bisacrylamide minigels for 35 min at 170 V, stained with ethidium bromide (1.0 μ g/mL), and visualized under UV light. The gels were photographed with a digital camera (DC40, Kodak, Rochester, NY) and analyzed with 1D Image Analysis software (Kodak, Rochester, NY). The alleles were identified taking into account the combination of restriction patterns (<http://www2.ri.bbsrc.ac.uk/bola/>) and designated according to the allele nomenclature proposed by van Eijk *et al.* (1992).

Population Genetic Analysis

Gene and genotypic frequencies were estimated by direct counting. Deviations from Hardy–Weinberg (HW) equilibrium were estimated by the F_{IS} parameter (Weir and Cockerham, 1984). The significance of this statistic was measured by the Markov Chain method (Gou and Thompson, 1992). The amount of gene diversity in the studied breed was measured by the number of alleles (n_a) and the unbiased expected heterozygosity (h_e) according to the formula proposed by Nei and Roychoudhury (1974). The Ewens–Watterson–Slatkin exact neutrality test was estimated using the method described by Slatkin (1996). The Arlequin 2.0 program (Schneider *et al.*, 2000) was used to estimate the parameters mentioned above.

RESULTS AND DISCUSSION

At population levels, considerable gene frequency data are available for *BoLA-DRB3* (Aravindakshan and Nainar, 1999; Dietz *et al.*, 1997a,b; Gilliespie *et al.*, 1999; Giovambattista *et al.*, 1996; Maillard *et al.*, 1999; Sharif *et al.*, 1998). These studies have involved highly selected breeds, such as Holstein, Black Pied, Ayrshire, and Jersey (Dietz *et al.*, 1997a,b; Gilliespie *et al.*, 1999; Sharif *et al.*, 1998;

Table I. Gene Frequency Distribution of *BoLA-DRB3.2* Alleles Detected in Saavedreño Creole Dairy Cattle

Allele	Frequency	Allele	Frequency	Allele	Frequency
<i>DRB3.2*1</i>	0.0120	<i>DRB3.2*16</i>	0.1680	<i>DRB3.2*27</i>	0.0840
<i>DRB3.2*5</i>	0.0280	<i>DRB3.2*18</i>	0.0400	<i>DRB3.2*29</i>	0.0080
<i>DRB3.2*6</i>	0.0040	<i>DRB3.2*20</i>	0.0200	<i>DRB3.2*34</i>	0.0120
<i>DRB3.2*7</i>	0.0760	<i>DRB3.2*21</i>	0.0480	<i>DRB3.2*35</i>	0.0080
<i>DRB3.2*8</i>	0.0920	<i>DRB3.2*22</i>	0.0240	<i>DRB3.2*36</i>	0.1160
<i>DRB3.2*10</i>	0.0160	<i>DRB3.2*23</i>	0.0240	<i>DRB3.2*37</i>	0.0800
<i>DRB3.2*11</i>	0.0840	<i>DRB3.2*24</i>	0.0240		
<i>DRB3.2*15</i>	0.0240	<i>DRB3.2*25</i>	0.0080		

Udina *et al.*, 1998). Also, Argentine Creole and Martinique Brahman cattle breeds have been analyzed for this locus (Aravindakshan and Nainar, 1999; Giovambattista *et al.*, 1996; Maillard *et al.*, 1999).

In the present work, 22 out of the 53 previously reported *BoLA-DRB3.2* alleles were detected in the studied sample of Saavedreño Creole cattle. As shown in Table I, 70% of the cumulative gene frequencies corresponded to the seven most common alleles, which are defined as variants with gene frequencies higher than 5% (*DRB3.2*16*, *36, *8, *11, *27, *37, and *7). An additional 11 patterns were detected with frequencies between 1 and 5% (*DRB3.2*21*, *18, *5, *15, *22, *23, *24, *20, *10, *1, and *34), while the remaining 4 variants exhibited gene frequencies lower than 1% (*DRB3.2*25*, *29, *35, and *6).

Comparison between the present results with the allele spectrum and the gene frequencies profile previously reported in other cattle breeds showed the following. First, in the previously studied breeds, cumulative gene frequencies of the most frequent (>0.05) alleles varied from 70 to 88.7%, while the frequency of the most abundant variant ranged from 14.3 to 37.6%. Values in the Saavedreño Creole cattle were close to the lowest limits for both ranges (70 and 16.8% respectively; see Tables I and II). Second, the identified alleles as well as the composition of alleles with higher frequencies differed significantly between breeds (Table II). Saavedreño Creole cattle share one to four high-frequency alleles with the other previously studied cattle breeds.

As expected, the high number of alleles with appreciable gene frequencies detected in the Saavedreño Creole cattle resulted in a high value of heterozygosity (Table II). The high degree of *BoLA-DRB3.2* polymorphism (measured through n_a and h_e parameters) estimated for this breed is similar to those values previously reported in other bovine cattle breeds, such as Holstein, Jersey, Aberdeen Angus, Ayrshire, Black Pied, and Brahman (Dietz *et al.*, 1997a,b; Gilliespie *et al.*, 1999; Golijow, 1996; Maillard *et al.*, 1999; Sharif *et al.*, 1998; Udina *et al.*, 1998) (Table II). However, the h_e value seems to be quite high for Creole cattle

Table II. The Number of Alleles and the Value of Expected Heterozygosity Observed in the Saavedreño Creole, the Argentine Creole, Jersey, Holstein, and Martinique Brahman Cattle Breeds

Breed	n_a	h_e	n_a^a	% ^a	Alleles with gene frequency higher than 0.05	N
Saavedreño ^b	22	0.919	7	70.00	<i>DRB3.2</i> *16, *36, *8, *11, *27, *37, *7 ^b	125
Argentine ^c	21	0.870	6	72.80	<i>DRB3.2</i> *5, *15, *18, *20, *24, *27 ^c	194
Aberdeen Angus ^d	16	0.870	6	76.60	<i>DRB3.2</i> *36, *8, *4, *15, *22, *20, *10 ^d	65
Jersey ^{e,f}	24	0.895	7	79.40	<i>DRB3.2</i> *8, *10, *15, *17, *21, *36, *ibe ^f	172
	13	0.886	6	81.00	<i>DRB3.2</i> *7, *10, *17, *21, *28, *32 ^g	66
Holstein ^{e,g}	26	0.741	7	88.70	<i>DRB3.2</i> *3, *7, *8, *11, *16, *22, *23, *24,	1100
	27	0.872	7	76.20	*27 ^{e,g}	835
	24	0.932	7	64.40		115
Ayrshire ^h	18	0.821	5	77.10	<i>DRB3.2</i> *8, *7, *28, *8, *10, *24 ^h	129
Black Pied ^h	21	0.904	8	81.20	<i>DRB3.2</i> *22, *24, *11, *16, *18, *23, *8, *27 ^h	127
Brahman ⁱ	37	0.843	ND	ND	ND	568

Note. n_a = number of alleles, h_e = heterozygosity, N = sample size. ND = Not determined.

^a n_a and cumulative gene percentage (%) of alleles with gene frequency higher than 0.05.

^bPresent work.

^cGiovambattista *et al.* (1996).

^dGolijow (1996).

^eDietz *et al.* (1997a,b).

^fGilliespie *et al.* (1999).

^gSharif *et al.* (1998).

^hUdina *et al.* (1998).

ⁱMaillard *et al.* (1999).

(Giovambattista *et al.*, 1996), probably because the founder group was constituted with animals from different Creole cattle populations.

In spite of the small population size of Saavedreño Creole cattle ($n = 300$ adult animals) and artificial selection for dual-purpose production (dairy and beef) in this breed during the last 30 years, our results reveal that this breed exhibits a high degree of genetic variation at the *BoLA-DRB3* locus. A possible explanation for these results, as mentioned above, is the multiple origins of the founder population of Saavedreño Creole cattle, and the further maintenance of diversity through some selected mechanism. At least three mechanisms have been previously proposed, namely, reproductive mechanism, overdominance, and frequency-dependent selection.

The reproductive mechanisms include *MHC*-based mating preferences and selective abortion. They have been documented in both mice and human populations and may show sufficiently strong effects to maintain *MHC* diversity (Potts *et al.*, 1991; Wedekind and Furi, 1997). However, the extensive genetic diversity at *BoLA-DRB3* locus in the Saavedreño dairy cattle breed, as well as in other farm animals, cannot be explained by mating-type preferences, since the reproduction of this breed has been controlled by breeders since the herd was established.

The HW test showed that the studied population did not deviate significantly from the theoretical proportions ($F_{IS} = -0.026$; $p = 0.681$). Heterozygote deficit

or excess was tested in the "Saavedreño" population, and the results did not show any significant difference from zero (F_{IS} deficit = 0.009; $p = 0.084$, and F_{IS} excess = 0.023; $p = 0.871$). These results contrasted with data observed in larger populations, such as human, where a significant homozygote deficit has been reported for *MHC* genes (Black and Salzano, 1983; Boyce *et al.*, 1996; Markow *et al.*, 1993). The excess of heterozygosity has been interpreted as a consequence of overdominance, because heterozygous individuals are able to recognize a broader spectrum of foreign antigens, increasing their relative fitness when compared with homozygotes (Hedrick *et al.*, 1991; Hughes and Nei, 1989). The most likely explanation for the absence of heterozygote excess in the Saavedreño breed is that the overdominance selection coefficient at the *MHC* loci is quite low (probably lower than 0.02; Mikko *et al.*, 1999). Such weak selection would be enough to increase heterozygotes only in large populations, and in the absence of high rates of stochastic forces (population bottlenecks, genetic drift, inbreeding). For this reason, and because this method may suffer from low resolving power, such effects could not be observed in the Saavedreño population.

The neutrality test showed an even gene frequency distribution in spite of the HW equilibrium (Slatkin's Exact $p = 0.004$) as expected under balancing selection rather than allelic profiles expected under positive selection against one or few alleles, or under neutral selection. This result is expected if the sampled population is under relative recent balancing selection rather than positive or neutral selection. This finding has also been reported in other cattle breeds and ruminants. For example, Paterson *et al.* (1998) observed a very even allele frequency distribution at microsatellite markers within *MHC* in the Soay sheep population on St. Kilda (Scotland). Furthermore, Kantanen *et al.* (2000) reported that the microsatellite *BoLA-DRBP1*, located within the *BoLA* locus, is not neutral in several Scandinavian bovine breeds (Eastern Finncattle, Northern Finncattle, Doela cattle, Blackside Troender and Norland cattle, and Danish Shorthorn).

The even allele frequency distribution observed at *MHC* loci could be explained assuming that the presence of a particular allele rather than heterozygosity is the critical factor determining survivorship and fitness differences. If so, each *MHC* allele could be related to protection against a different infectious disease or associated with distinct fitness traits such as survival or fecundity. Up to now, different authors have reported several associations between a particular allele of the *BoLA* genes and resistance/susceptibility to some infectious diseases in cattle. For example, Schmutz *et al.* (1992) indicated that one *BoLA-DRB* gene pattern in a study of 106 Holstein cows was associated with resistance to *Staphylococcus aureus* mastitis. Associations between *BoLA* allele types and persistent lymphocytosis caused by bovine leukosis virus have also been reported (Lewin and Bernoco, 1986; Zanotti *et al.*, 1996). More recently, Sharif *et al.* (1998) indicated that the *BoLA-DRB3.2*3* allele was associated with a significantly lower risk of cystic ovarian disease in Holstein dairy cows.

IMPLICATIONS

The results provided in the present work underline the high degree of polymorphism at the *BoLA-DRB3* locus in the Saavedreño Creole dairy cattle breed. This variability could have been caused by the multiple origins of the herd and further maintained through balancing selection. In addition, present data confirmed the existence of differences in the allelic frequency distribution and allelic composition within and among domestic cattle breeds for the studied locus. Taking into account cumulative frequencies, frequencies of the most abundant variant, and the neutrality test, the gene frequency distribution of Saavedreño Creole seems to be slightly more even in comparison with that described for other cattle breeds. However, the inference suggested in the present work about the effect of balancing selection on *BoLA-DRB3.2* gene frequency distribution has to be confirmed with additional studies. The analysis of *MHC* polymorphism in tropical dairy breeds, like Saavedreño Creole cattle, is important for dairy and industry. The relationship of the *MHC* locus with fitness and resistance/susceptibility to disease could be useful information to increase dairy production in tropical regions where European dairy breeds perform poorly.

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REFERENCES

- Aravindakshan, T. V., and Nainar, A. M. (1999). Genetic polymorphism of the *BoLA-DRB3* gene in Jersey crossbred and Ongole cattle revealed by PCR-RFLP. *J. Anim. Sci.* **69**:424–427.
- Black, F. L., and Salzano, F. M. (1983). Evidence for heterosis in the HLA system. *Am. J. Hum. Genet.* **33**:894–899.
- Boyce, W. M., Hedrick, P. W., Muggli-Cockett, N. E., Kalinowski, S., Penedo, M. C. T., and Ramey, R., II (1996). Genetic variation of major histocompatibility complex and microsatellite loci: A comparison in Bighorn sheep. *Genetics* **145**:421–433.
- Burke, M. G., Stone, R. T., and Muggli-Cockett, N. E. (1991). Nucleotide sequence and northern analysis of a bovine major histocompatibility class II DR β -like cDNA. *Anim. Genet.* **22**:343–352.
- Dietz, A. B., Cohen, N. D., Timms, L., and Kehrl, M. E., Jr. (1997a). Bovine lymphocyte antigen class II alleles as risk factors for high somatic cell counts in milk lactating dairy cows. *J. Dairy Sci.* **80**:406–412.
- Dietz, A. B., Detilleux, J. C., Freeman, A. E., Kelly, D. H., Stabel, J. R., and Kehrl, M. E., Jr. (1997b). Genetic association of bovine lymphocyte antigen DRB3 alleles with immunological traits of Holstein cattle. *J. Dairy Sci.* **80**:400–405.
- Ellis, S. A., and Ballingall, K. T. (1999). Cattle MHC: Evolution in action? *Immunol. Rev.* **167**:159–168.

- Gilliespie, B. E., Jayarao, B. M., Dowlen, H. H., and 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., Golijow, C. D., Dulout, F. N., and Lojo, M. M. (1996). Gene frequencies of *DRB3.2* locus of Argentine Creole cattle. *Anim. Genet.* **27**:55–56.
- Golijow, C. D. (1996). *Estudio de la reducción de la variabilidad genética por acción de la selección artificial en poblaciones de Bos taurus*, PhD Thesis, Universidad Nacional de La Plata, Argentina.
- Gou, S. W., and Thompson, E. A. (1992). Performing the exact test of Hardy–Weinberg proportions for multiple alleles. *Biometrics* **48**:361–372.
- Hedrick, P. W. (1994). Evolutionary genetics of the major histocompatibility complex. *Am. Nat.* **143**:945–964.
- Hedrick, P. W., Whittam, T. S., and Parham, P. (1991). Heterozygosity at individual amino acid sites: Extremely high levels for HLA-A and HLA-B genes. *Proc. Natl. Acad. Sci.* **88**:5897–5901.
- Hughes, A. L., and Nei, M. (1989). Nucleotide substitution at major histocompatibility complex class II loci: Evidence for over-dominant selection. *Proc. Natl. Acad. Sci. U.S.A.* **86**:958–962.
- Kantanen, J., Olsaker, I., Holm, L. E., Lien, S., Vilkki, J., Brusgaard K., Eythorsdottir, E., Danell, B., and Adalsteinsson, S. (2000). Genetic diversity and population structure of 20 north European cattle breeds. *J. Hered.* **91**(6):446–457.
- Klein J. (1986). *Natural History of the Major Histocompatibility Complex*, Wiley, New York.
- Lewin, H. A. (1994). Host genetic mechanism of resistance and susceptibility to a bovine retroviral infection. *Anim. Biotechnol.* **5**:183–191.
- Lewin, H. A., and Bernoco, D. (1986). Evidence for *BoLA*-linked resistance and susceptibility to subclinical progression of bovine leukaemia virus infection. *Anim. Genet.* **17**:197–207.
- Maillard, J. C., Renard, C., Chardon, P., Chantal, Y., and Bensaid, A. (1999). Characterization of 18 new *BoLA-DRB3* alleles. *Anim. Genet.* **30**:200–203.
- Markow, T., Hedrick, P. W., Zuerlein, K., Danilows, J., Martin, J., Vyvial, T., and Armstrong, C. (1993). HLA polymorphism in the Havasupai: Evidence for balancing selection. *Am. J. Hum. Genet.* **53**:943–952.
- Mikko, S., Røed, K., Schmutz, S., and Andersson, L. (1999). Monomorphism and polymorphism at *MCH DRB* loci in domestic and wild ruminants. *Immunol. Rev.* **167**:169–178.
- Nei, M., and Roychoudhury, A. K. (1974). Sampling variances of heterozygosity and genetic distance. *Genetics* **76**:379–390.
- Paterson, S., Wilson, K., and Pemberton, J. M. (1998). Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population (*Ovis aries* L.). *Proc. Natl. Acad. Sci. U.S.A.* **95**:3714–3719.
- Potts, W. K., Manning, C. J., and Wakeland, E. K. (1991). MHC genotype influences mating patterns in semi-natural populations of Mus. *Nature (Lond.)* **354**:453.
- Schmutz, S. M., Berryere, T. G., Robbins, J. W., and Carruthers, T. D. (1992). Resistance to *Staphylococcus aureus* mastitis detected by DNA markers. In *Proc. 31st Annu. Mtg. Natl. Mastitis Council, Arlington, VA., Natl. Mastitis Council, Inc., Madison, WI*, pp. 124–133.
- Schneider, S., Roessli, D., and Excoffier, L. (2000). *Arlequin: a Software for Population Genetic Data Analysis*, Ver. 2.000, University of Geneva, Geneva.
- Sharif, S., Mallard, B. A., Wilkie, B. N., Sardeant, J. M., Scott, H. M., Dekkers, J. C. M., and 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.
- Slatkin, M. A. (1996). Correction to the exact test based on the Ewens sampling distribution. *Genet. Res.* **68**:259–260.
- Udina, I. G., Haramyshera, E. E., Sulimova, G. E., Pavlenko, S. P., Turkova, S. O., Orlova, A. R., and Ernst, L. K. (1998). Comparative analysis of Ayrshire and Black Pied cattle breeds by histocompatibility markers. *Genetika* **34**(12):1668–1674.
- van Eijk, M. J. T., Stewart-Haynes, J. A., and Lewin, H. A. (1992). Extensive polymorphism of the *BoLA-DRB3* gene distinguished by PCR-RFLP. *Anim. Genet.* **23**:483–496.
- Wedekind, C., and Furi, S. (1997). Body odour preferences in men and women: Do they aim for specific MHC combinations or simply heterozygosity? *Proc. R. Soc. Lond. B. Biol. Sci.* **264**(1387):1471–9.

- Weir, B. C., and Cockerham, C. C. (1984). Estimating F -statistics for the analysis of population structure. *Evolution* **38**:1358–1370.
- Zanotti, M., Poli, G., Ponti, W., Polli, M., Rocchi, M., Bolzani, E., Longeri, M., Russo, S., Lewin, H. A., and van Eijk, M. J. T. (1996). Association of BoLA class II haplotypes with subclinical progression of bovine leukaemia virus infection in Holstein–Friesian cattle. *Anim. Genet.* **27**:337–341.