Characterization of major histocompatibility complex DRB diversity in the endemic South African antelope *Damaliscus pygargus*: a comparison in two subspecies with different demographic histories

J. M. VAN DER WALT*, L. H. NEL* and A. R. HOELZEL†

*Department of Microbiology and Plant Pathology, University of Pretoria, Republic of South Africa 0002, †Department of Biological Sciences, University of Durham, Durham, UK

Abstract

Major histocompatibility complex (MHC) class II locus DRB was investigated by single-strand conformation polymorphism analysis (SSCP) and sequence analysis in the endemic South African antelope, *Damaliscus pygargus*, of which there are two subspecies. Greater polymorphism was found in the blesbok (*D. p. phillipsi*) subspecies (*n* = 44; 22 alleles) than in the bontebok (*D. p. pygargus*) subspecies (*n* = 45; 6 alleles). Erosion of allelic diversity in bontebok was most likely the result of two severe bottleneck events caused by hunting pressure and parasitic infection. A majority of the polymorphism observed was found within the peptide binding region (PBR) where dN/dS ratios were higher than for the non-PBR region. This, and the apparent trans-species relationship among alleles in a bovid phylogeny, suggest the evolution of diversity by heterosis or frequency-dependent selection.

Keywords: antelope, bottleneck, class II MHC, DRB, evolution, trans-species

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Introduction

Major histocompatibility complex (MHC) class II loci encode cell-surface glycoproteins on antigen-presenting cells that function to bind and present foreign peptides to the immune system (Klein 1986). The genes that encode the MHC protein receptors are the most polymorphic loci of all nuclear-encoding genes in vertebrate species (Hughes & Hughes 1995). Studies on the evolution of MHC genes, particularly those of domestic mammals and humans, have shown extreme polymorphism, maintenance of diversity by frequency-dependent selection or heterosis, and a trans-species mode of evolution (Doherty & Zinkernagel 1975; Klein 1987; Hughes & Nei 1989). The evidence for selection is based in part on the observation that nonsynonymous substitutions are significantly more common than synonymous substitutions at functional residues in these loci (Hughes & Nei 1989; Takahata & Nei 1990). The forces of balancing selection act to maintain ancient alleles and allele motifs in a trans-specific manner.

Correspondence: J. M. van der Walt. Fax: 27-12-420-3622; E-mail: mwen624285@aol.com

Trans-specific evolution refers to polymorphism that predates speciation events, whereby allelic lineages are passed from species to species and persist over long periods of evolutionary time (Klein 1987).

Recently, these genes have been investigated to assess genetic diversity in populations of exotic species. High allelic diversity has been documented in MHC genes in most outbred species (Hedrick 1994). However, MHC diversity can be lost through demographic crashes, as documented in populations of northern elephant seal (Hoelzel *et al*. 1999) and cheetah (Yuhki & O'Brien 1997), or by isolation on islands, as reported in Australian bush rats (Seddon & Baverstock 1999). In order to further our understanding of how demography and environment influence MHC evolution in wild populations, we chose to study the DRB homologue in the endemic South African antelope, *Damaliscus pygargus*.

D. pygargus belongs to the Alcelaphini tribe which, according to the fossil record, arose ≈ 5 Ma in South Africa and spread throughout the African continent (Vrba 1979). This tribe comprises the following extant species: the tsessebe or topi (*Damaliscus lunatus*), hartebeest (*Alcelaphus* sp.) and wildebeest (*Connochaetes* sp.). Historically, *D. pygargus* had
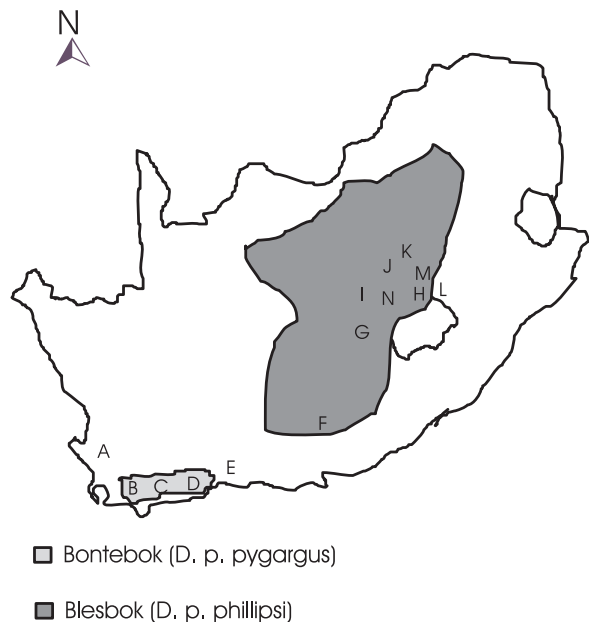
Fig. 1 Map of the natural distribution of blesbok and bontebok antelope throughout South Africa. Shaded areas represent the allopatric range for each subspecies. Sampling locations and number of animals genotyped are as follows: A = West Coast National Park (10), B = Overberg Farm (8), C = Bontebok National Park (14), D = Heidleberg Farm (2), E = Elandsberg Farm (4), F = Cradock (2), G = De Brug (2), H = Golden Gate National Park (3), I = Bloemhof Farm (3), J = Parys Farm (25), K = Suikerbosrand Reserve (2), L = Sterkfontein Dam Reserve (2), M = TDR Farm (5), and N = Fairview Farm (2).

a continuous distribution covering the south-western Cape region of South Africa to the southern boundary of Zimbabwe and did not extend as far north as its conspecifics (Vrba 1979). The species was divided by climatic and habitat changes into two groups (blesbok: *D. p. phillipsi* and bontebok: *D. p. pygargus*) and has since remained allopatric for hundreds of thousands of years (Skead 1980; Skinner & Smithers 1990) (Fig. 1). This isolation has allowed morphological differences such as coat colour and body markings to arise in each group (Bigalke 1955). The common names have been entrenched for over 300 years and are used here to represent each subspecies.

Historically, bontebok were restricted to the coastal plains of the western Cape region. European settlement within the Cape began in 1652 and occupied the most fertile land for agriculture. Intensive human encroachment and strong hunting pressure attributed to the near extinction of the endemic South African bontebok by the early 1800s (van der Merwe 1968). A relic population (*n* = 27) was protected on farmland, which in turn, led to the slow recovery of this antelope. In 1931, a herd from this population (*n* = 17) was translocated to the first Bontebok National Park (BNP) which was proclaimed for the protection of bontebok from

further hunting pressure (Bigalke 1955). Three decades later, a large mortality (50%) resulted within this herd from massive worm infestations of conical fluke (*Paramphistomum* sp.), lung worm (*Protostrongylus* sp.), wireworm (*Haemonchus* sp.), brown stomach worm (*Ostertogia* sp.) and bankrupt-worm (*Tirchostrongulus* sp.), as well as copper deficiency and related syndromes. Surviving animals (*n* = 61) were later translocated to a newly established BNP in 1960 (Barnard & van der Walt 1961). The estimated 2500–3000 bontebok today are derived from this founding population (Warden Fourie, BNP, personal communication). Bontebok are still considered a rare antelope species and are listed as vulnerable in Appendix II of the International Trade in Endangered Species Red List (World Conservation Union 1993).

Blesbok roamed the grasslands of the Eastern Cape, Free State (former Orange Free State), Gauteng and Mpumalanga (former Transvaal) (Skinner & Smithers 1990). These antelope were also persecuted by early settlers for their hides and were reported as having been slaughtered by the thousands after the Great Trek in 1836. The naturalist H. A. Bryden (1893) wrote 'The blesbok which not long since scoured the plains in the countless thousands, is now seldom seen'. The extermination of blesbok populations persisted until 1899 when the trade in skins was brought to an end by the Boer War. After the war, the majority of the open grassland regions were taken over by settlers forcing the remaining populations to exist only on fenced farms. By 1962, the population size of the South African blesbok was estimated at being ≈ 46 000 antelope (Kettlitz 1967). Blesbok are still a popular game antelope today, although hunting is now confined to private lands and national hunting concessions. The current population size of blesbok is ≈ 120 000 (David 1997).

Our aims were to characterize the pattern of polymorphism at the DRB locus in the exotic African bovid species, *D. pygargus*, and to assess the impact of population bottlenecks on diversity at this locus in one of two subspecies. We expect to find less DRB polymorphism in the bontebok subspecies, given its past demographic history. Variation at the sequence level was investigated to define the regions of functional significance involved in antigen presentation. Levels of allelic diversity were estimated in each subspecies to assess the effect of population collapse on a non-neutral locus. This locus was chosen as a marker due to its highly polymorphic nature in cattle and outbred species (Ellis & Ballingall 1999; Lewin *et al.* 1999). DRB sequences derived from distantly related ungulate species were aligned with those of *D. pygargus* (herein referred to as Dama) so that the mode of evolution and relationships of alleles could be revealed in a phylogenetic analysis. This study presents novel DRB alleles and provides evidence for mechanisms of trans-species evolution and balancing selection acting on the DRB locus in a new species.

Materials and methods

Samples and collection sites

A total of 45 bontebok and 44 blesbok samples was collected from private farms and national parks throughout South Africa (Fig. 1). A majority of the bontebok samples (blood and ear tissue) were taken during 1994–99 from live animals that had been chosen for translocation events. Blesbok tissue samples were derived from herd culls (liver and heart) or live animals (blood) during captures from 1996 to 1999. Sampling also included material from hybrid animals (*Bb1-2*) from a private farm (Fairview) located in the Free State. Total genomic DNA was extracted from bontebok and blesbok antelope samples using standard methods.

Polymerase chain reaction — single-strand conformation polymorphism

Cattle-specific primers HL030 and HL031 were used to amplify a 284-bp fragment of exon 2 of the DRB locus from genomic DNA as described by Van Eijk *et al.* (1992). Polymerase chain reaction (PCR) products (8 µL) were mixed with 32 µL of a low ionic strength buffer (LIS) and subjected to heat denaturation at 97 °C for 2 min. The single strands were formed within the LIS sugar matrix (10% saccharose, 0.01% bromophenol blue, 0.01% xylene cyanol FF) and remained stable at room temperature (Maruya *et al.* 1996).

Single-stranded products were then subjected to electrophoresis through a 10% nondenaturing polyacrylamide gel (1.4% cross-linking) in 1× TBE (Tris borate EDTA) buffer at room temperature for 18 h (Glavac & Dean 1993). After electrophoresis, the gel was incubated for 30 min with the fluorescent GelStar Nucleic Acid Gel Stain (BioWhittaker). Alleles were visualized by UV illumination and image capture with a Kodak digital camera. Allele patterns were scored for each animal and genotypes assigned. Unique patterns of allelic variation were investigated by sequence analysis. In most cases, gel fragments were excised and purified for sequencing (Sambrook *et al.* 1989).

Cloning and sequencing

A subset of alleles as revealed by single-strand conformation polymorphism analysis (SSCP) mobilities were chosen for cloning and sequencing. Homozygous individuals were sequenced directly from purified DRB PCR products. Heterozygous genotypes were cloned into a pGEM vector (Promega) using a TA cloning kit. Ten recombinant clones were purified by mini-prep filter columns (Qiagen) and sequenced in the forward and reverse directions using the BigDye Terminator Cycle Sequencing Ready Reaction Kit

(ABI). The labelled PCR fragments were analysed using an ABI 377 automated sequencer.

Statistical and phylogenetic analyses

All Dama-DRB nucleotide sequences and one cow reference sequence (*DRB3*0101*) were aligned and translated into the corresponding amino acid sequences using the MEGA software package (Groenen *et al.* 1990; Kumar *et al.* 1993). Amino acid positions involved in peptide binding were identified by comparison with the peptide binding groove structure of the human class II molecule (Brown *et al.* 1993). Relative frequencies of nonsynonymous (dN) and synonymous (dS) substitutions were calculated for the peptide binding region (PBR) and non-PBR according Nei & Gojobori (1986) using the Jukes–Cantor (1969) correction. All dN and dS frequencies plus standard errors were estimated using the MEGA computer package. Significance levels were determined by the Student's t-test with an infinite degrees of freedom, where $t = d/s(d)$ (Kumar *et al.* 1993). Heterozygosity levels were determined assuming random mating within subpopulations and deviation from the Hardy–Weinberg equilibrium was tested using Markov chain permutation test of 100 000 steps in GENEPOP version 1.2 (Raymond & Rousset 1995). Genetic distance matrices were estimated using the Hasegawa–Kashino–Yano (HKY) model (Hasegawa *et al.* 1985) and the Kimura 2-parameter model Kimura (1980). Phylogenetic relationships among alleles were estimated by both minimum evolution (neighbour-joining) and maximum likelihood methods using PAUP*, version 4.0b1 (Swofford 1998).

GenBank database sequences for phylogenetic analysis

Cattle sequences representing 39 of the major BLA allelic lineages were acquired through the BoLA nomenclature Website: http://www2.ri.bbsrc.ac.uk.bola/) and used as in a phylogenetic analysis of cattle DRB exon 2 sequences: AF144544, AF144545, Aj001999, Aj002001, U77067, U78548, X87643–X87645, X87647–X87650, X87652, X87655, X87656, X87658, X87659, X87662, X87665–X87668, X87670, Z30650, Z30649, Z36538, Z36542, Z82023–Z82025, Z82027, Z82029, Z82032–Z82035. DRB sequences derived from horse, *ELA-DRB*9* (L76974) and moose, *ALA1DRB1*8* (X83284) were used as outgroup taxa for the phylogenetic analyses.

Results

The Dama-DRB homologue to the cow *BoLA-DRB3* locus was characterized by SSCP analysis followed by cloning and direct sequencing of alleles. We are confident that only one DRB locus was amplified as no more than two sequences were revealed in each animal. Cloned PCR products revealed only two alleles through sequence

Table 1 DRB allele frequency distribution

DRB allele	Blesbok	Bontebok
Dama*01	0.204	0.000
Dama*02	0.113	0.000
Dama*03	0.068	0.000
Dama*04	0.170	0.000
Dama*05	0.011	0.000
Dama*06	0.023	0.000
Dama*07	0.011	0.090
Dama*08	0.068	0.390
Dama*09	0.011	0.000
Dama*10	0.011	0.000
Dama*11	0.011	0.000
Dama*12	0.011	0.000
Dama*13	0.011	0.000
Dama*14	0.000	0.344
Dama*15	0.079	0.000
Dama*16	0.000	0.067
Dama*17	0.034	0.000
Dama*18	0.011	0.000
Dama*19	0.045	0.000
Dama*20	0.034	0.000
Dama*21	0.011	0.000
Dama*22	0.000	0.033
Dama*23	0.000	0.078
Dama*24	0.011	0.000
Dama*25	0.011	0.000
Dama*26	0.023	0.000
Dama*27	0.011	0.000

analysis of a minimum of eight recombinant clones for any heterozygote. Alignment of all Dama alleles demonstrates close homology to the cattle *BoLA-DRB3* expressed locus. This close homology and the absence of stop codons within the nucleotide sequences provide evidence that the expressed DRB locus was isolated in *Damaliscus pygargus*. Sequence variation of exon 2 was examined by SSCP revealing 27 different alleles. Allelic frequencies are given in Table 1. Extensive polymorphism was found for the blesbok subspecies (22 alleles) compared with the level of polymorphism found for the bontebok subspecies (six alleles). Two alleles are shared between subspecies (*Dama*7* and *Dama*8*). The sample Bb1 from the Fairview hybrid population displayed alleles from both subspecies. Heterozygosity values are shown in Table 3.

Polymorphism at class II loci occurs predominately in exon 2, which encodes a majority of the peptide region (PBR). The exon translates into a sequence length of 83 amino acids with 16 possible binding sites for foreign peptide presentation (Brown *et al.* 1993). In an alignment of all inferred exon amino acid sequences, 39 sites (47.0%) were variable, while the other sites were conserved. The majority of amino acid site changes were found within the PBR or in positions that are adjacent to these binding sites,
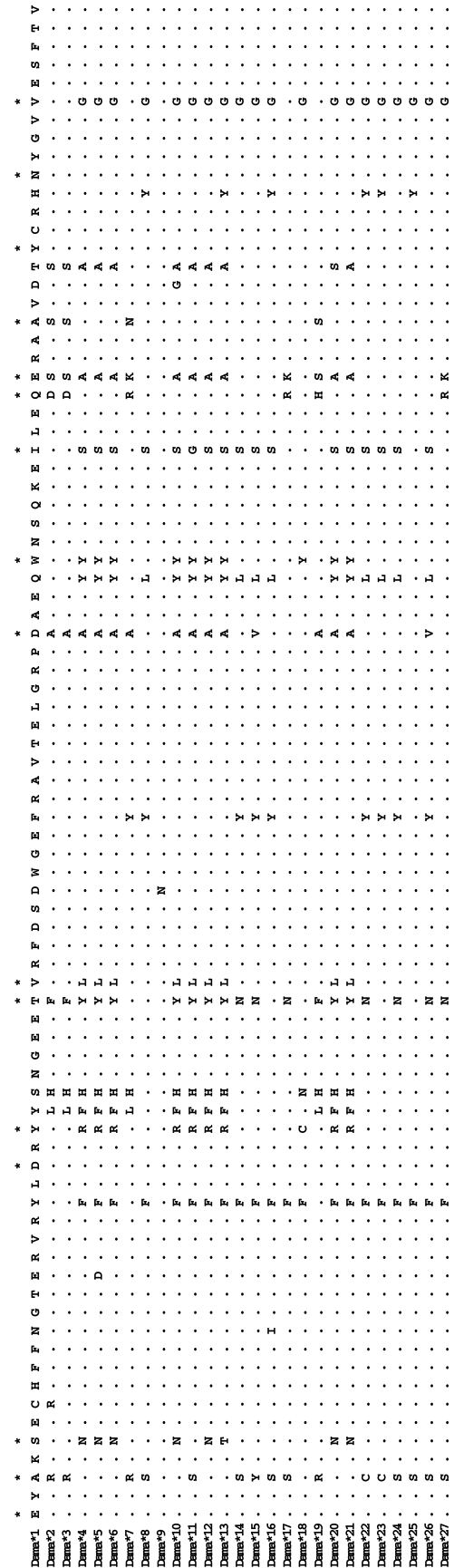

Fig. 2 Alignment of amino acid sequences from the 27 DRB alleles of *Damaliscus pygargus*. Sequence similarity to *Dama*1* is indicated by a dot while amino acid changes are represented by single letter codes. An asterisk indicates the putative sites involved in peptide binding (Brown *et al.* 1993).

Subspecies	N	No. of alleles	D	H_E	H_O
Blesbok	44	22	10.7 (2.5)	0.89	0.82
Bontebok	45	6	5.0 (1.6)	0.71	0.51

Table 2 Allelic diversity values estimated for blesbok and bontebok

N = total number of each subspecies samples. D = % average genetic distance between alleles (Jukes-Cantor) with standard errors. H_E = expected heterozygosity (Nei 1987) and H_O = observed heterozygosity.

Exon 2	PBR dN	PBR dS	P-value	Non-PBR dN	Non-PBR dS	P-value
Full length	29.8 (6.6)	4.7 (4.0)	**	4.9 (1.0)	1.9 (1.1)	ns
β sheet	32.1 (11.1)	1.9 (2.7)	**	5.4 (1.4)	2.3 (1.9)	ns
α?helix	32.8 (11.6)	8.0 (8.3)	*	3.0 (1.6)	0.5 (0.5)	ns

Table 3 Jukes-Cantor corrected proportion of dS and dN substitutions

Significance values were estimated using the Student's t-test and are indicated by *$P < 0.1$, **$P < 0.05$, and ns (nonsignificant).

Species	No. of alleles	PBR dN	PBR dS	Non-PBR dN	Non-PBR dS	P-value
Cattle	68	41.1 (5.1)	13.0 (6.6)	4.4 (0.7)	2.2 (1.2)	< 0.001
Sheep	20	29.8 (5.6)	5.2 (5.5)	3.1 (0.6)	1.5 (0.7)	< 0.01
Moose	10	11.3 (3.2)	0	0	1.1 (1.1)	< 0.001
Roe deer	3	23.5 (7.4)	2.5 (4.6)	1.8 (0.9)	2.8 (2.0)	< 0.05
Reindeer	11	28.0 (5.9)	9.8 (7.4)	3.9 (1.0)	3.1 (1.7)	< 0.05
Red deer	49*	42.7 (5.5)	10.5 (4.8)	6.4 (0.9)	6.0 (1.8)	< 0.001
Blesbok/Bontebok	27	29.9 (6.6)	4.7 (4.0)	4.9 (1.0)	1.9 (1.1)	< 0.01

Table 4 Species comparison of exon 2 diversity within homologous DRB loci

*Alleles derived from more than one locus. Adapted from Mikko *et al*. 1999.

such as sites 23, 24 and 52 (Fig. 2). The relative frequency of nonsynonymous substitutions (dN = 0.299; SE = 0.066) was significantly higher than the frequency of synonymous substitutions (dS = 0.047; SE = 0.040) in the PBR for all alleles (Table 2). In the non-PBR region, substitutions were found to occur much less frequently (dN = 0.049; SE = 0.011: dS = 0.019; SE = 0.012).

The number of replacement sites in the α-helix (positions 174–250) of exon 2 was nearly equal to that of the β-sheet (positions 1–168) (see Table 3). Moreover, the number of synonymous changes within exon 2 of Dama alleles falls far below the rate substitution of cattle and most other ruminants (Mikko *et al*. 1999). This finding suggests that diversity of Dama alleles has evolved more recently than that of cattle and other ruminant species (Table 4).

Neighbour-joining (minimum evolution) and maximum likelihood trees based on 249 bp of exon 2 were constructed using Dama alleles and outgroup sequences derived from horse (Fraser & Bailey 1996) and moose (Mikko & Andersson 1995). Genetic distances were estimated using the HKY model (Hasegawa *et al*. 1985) and the Kimura 2-parameter model (Kimura *et al*. 1980) for the minimum evolution (ME) analysis. Both distance measures produced strongly supported trees of equal topology revealing three evolutionary lineages (1, 2, 3) of Dama alleles (Fig. 3). The phylogeny constructed from the maximum likelihood estimation recapitulates this pattern (data not shown). Most of the alleles found in the bontebok subspecies cluster together in lineage 1 (*Dama*8, *Dama*14, *Dama*16, *Dama*22, *Dama*23), while *Dama*7 clusters in lineage 3.

The relationship between Dama and domestic cow (*Bos indicus* and *Bos taurus* = *BoLA-DRB*) alleles was investigated in a second phylogenetic (ME) analysis (Fig. 4). In this analysis, sequences representing 39 major BoLA lineages display strong homology to the Dama alleles. The clustering of alleles from all three species displays the trans-species mode of evolution in which all alleles from Bovidae form one lineage (Klein 1986). However, Dama alleles cluster strongly together in a species-specific manner, which is indicative of the large time divergence between these species.
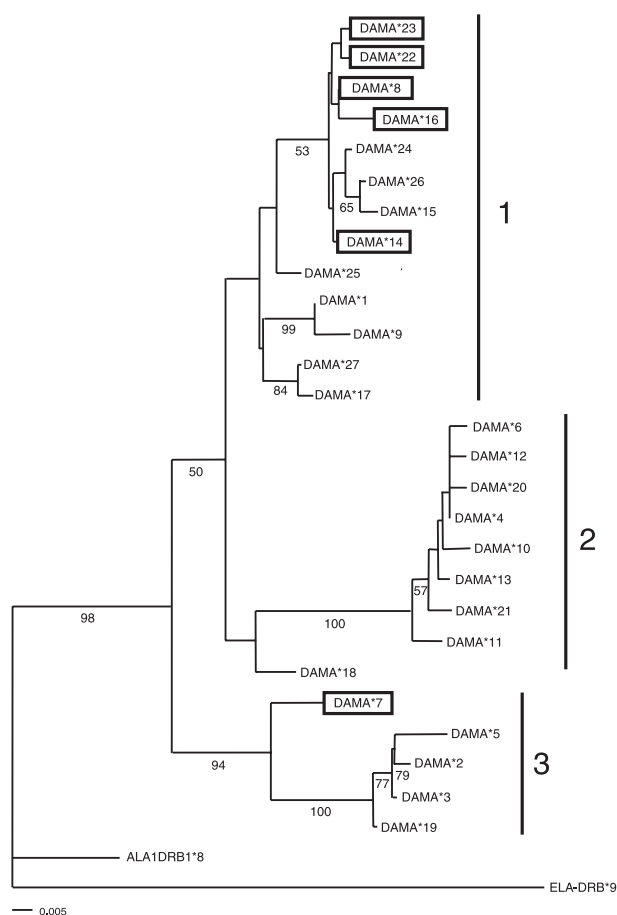
Fig. 3 Minimum evolution (ME) tree of Dama alleles rooted by horse and moose (*DRB1*9*) and horse (*ELA-DRB*8*) sequences. The phylogeny displays three evolutionary lineages within *Damaliscus pygargus*. Bontebok alleles are indicated by boxes.

Discussion

The DRB3 homologue was characterized in *Damaliscus pygargus* in order to study the evolution of this MHC class II locus in an exotic species of bovid, and to reveal the impact of population collapse on DRB3 polymorphism in the bontebok subspecies. In this study, we presented 27 novel ungulate DRB alleles from a threatened species of antelope that is distantly related to the cow. The relative levels of allelic diversity in the two subspecies were consistent with their different demographic histories as well as their geographical subdivision. Furthermore, this investigation provides evidence for the mechanisms of trans-species evolution and balancing selection acting on the DRB locus in a new exotic species.

The allelic diversity of each subspecies demonstrates a significant amount of genetic substructure within the species. Two alleles (*Dama*7* and *Dama*8*) were shared between the subspecies, whereas all others (*n* = 25) were private to each subspecies. MHC variation in chinook

salmon runs (Kim *et al.* 1999) and beluga whale populations (Murry *et al.* 1999) have also been found to be significantly differentiated. The genetic differences at this locus can be used to differentiate between bontebok and blesbok subspecies as well as identify hybrid animals. In one case, blesbok and bontebok alleles were found in a hybrid population indicating that at some point there had been genetic admixture in that herd.

There are several possible explanations for the robust structuring of the DRB locus, an unexpected result given the more typical trans-specific pattern of evolution observed for MHC loci (see Klein 1987; below). First, private alleles in one subspecies may be present in the other but failed to be sampled in this analysis. However, the sample size in this study was adequate (178 chromosomes examined) and the populations sampled were representative of the species distribution across South Africa. Of course, extensive sampling could ultimately uncover more shared alleles as well as additional novel alleles. Second, the partitioning of DRB alleles may represent the phylogeography of the species as bontebok and blesbok populations have been separated by the expanse of the Karoo Desert. Some lineage sorting could have occurred in these geographically isolated populations as a result of genetic drift, especially given the population contractions experienced by the bontebok subspecies (cf. Yuhki & O'Brien 1990). This scenario is probably the most likely, given the relatively small number of alleles found in bontebok. Some novel alleles may represent new mutations, possibly maintained by positive heterotic selection, although given the relevant time frame this could only explain a small proportion of the differences. Lastly, the private alleles may have had adaptive value to the subspecies and signify historic selective events. Because the bontebok subspecies experienced a second bottleneck event that was caused partly by parasitic infection, certain alleles may have been selected in response to presentation of the foreign antigen (Hamilton 1980).

A comparison of amino acid sequences showed extensive polymorphism within the PBR. The relative frequencies of nonsynonymous substitutions exceed that of synonymous substitutions in the PBR, suggesting that positive selection has maintained the allelic polymorphism in this species (Hughes & Nei 1989). The number of replacement sites in the α-helix (positions 174–250) of exon 2 was compared with that of the β-sheet (positions 1–168) in order to define the region in which selection pressure is greatest (Schwaiger *et al.* 1993). The similar values indicated that selection pressure is almost equal in both sequence regions. Moreover, the bovid phylogeny illustrates the conservation of ancestral polymorphisms that could not exist under a neutral model of evolution (Takahata & Nei 1990). The close homology of Dama alleles to cattle sequences reflects the transpecies mode of evolution of this MHC locus, which has been
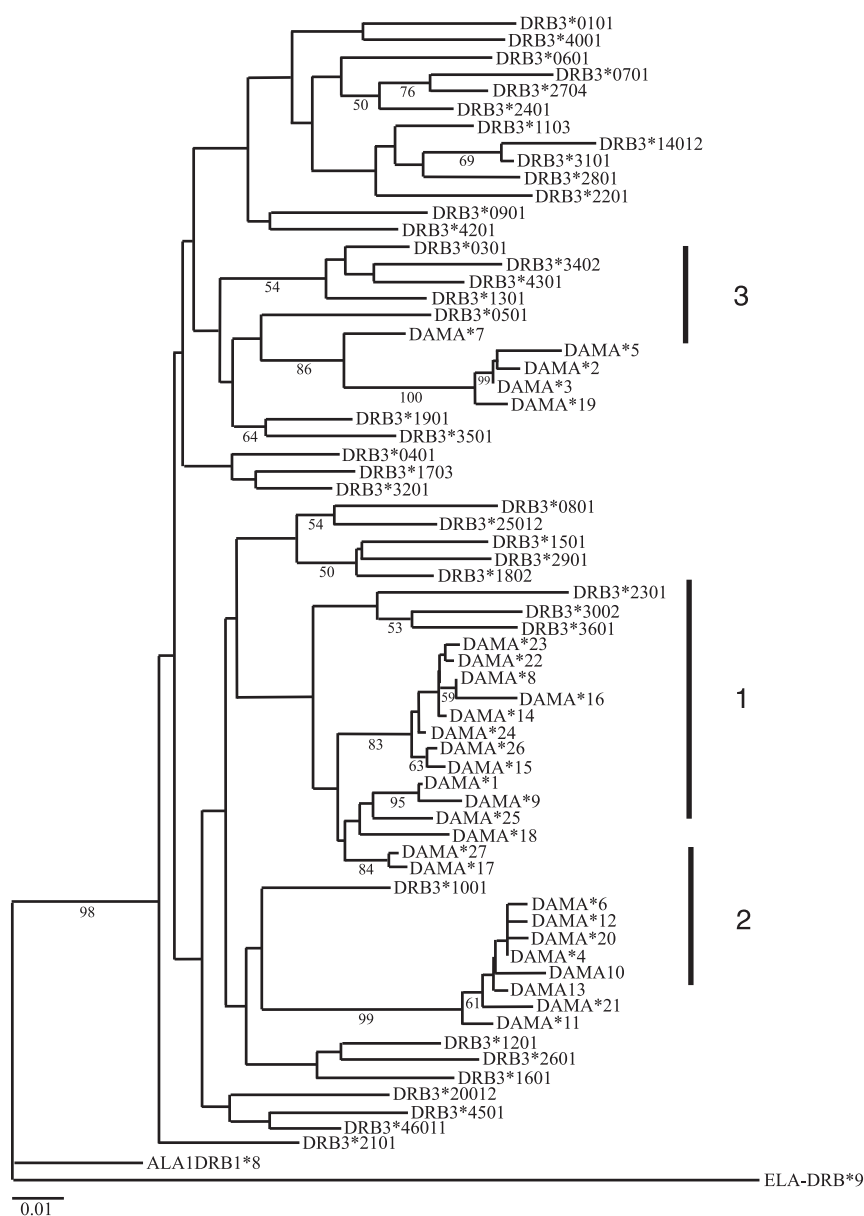
Fig. 4 Trans-species mode of evolution. The phylogeny reveals interleaving of bovid alleles indicating persistence of ancestral polymorphism within Bovidae. Strong species clustering of Dama alleles is also demonstrated (numbered lineages correspond to Fig. 3).

documented in primates (Klein *et al.* 1993), ungulates (Mikko *et al.* 1999), felines (Yuhki & O'Brien 1997), pinnepeds (Hoelzel *et al.* 1999) and rodents (Figuera *et al.* 1988). Mechanisms responsible for retaining polymorphism at MHC loci include overdominance, frequency-dependent selection (pathogen-driven) and disassortative (nonrandom) mating (Potts *et al.* 1991). It is generally assumed that allelic diversity at class II MHC genes evolves to promote pathogen resistance (Doherty & Zinkernagel 1975; O'Brien & Evermann 1988). This pattern of diversity, displaying elevated numbers of heterozygotes for polymorphic MHC class II genes, has been well documented in many outbred species and in humans (Markow *et al.* 1993; Hedrick 1994).

Disassortative (nonrandom) mating has been proposed to play a role in the selection of alleles whereby animals with dissimilar genotypes mate in order to avoid inbreeding within the population (Potts *et al.* 1994). Mate selection according to MHC genotypes has been documented in several vertebrate species (see Jordon & Bruford 1998 for review); however, MHC-based mating patterns have been excluded as a mechanism for generating allelic diversity in Soay sheep (Paterson & Pemberton 1997). There is no evidence to describe disassortive mating in bontebok, and other authors question the capacity for this selective force to account for the large number of amino acid site changes found within the PBR (Yeager & Hughes 1999). It is most likely that a combination of evolutionary mechanisms has

contributed to the enrichment of diversity found within both *Damaliscus* subspecies.

The phylogeny of full-length exon 2 Dama alleles revealed three distinct lineages of the DRB locus in this species. Alleles of each lineage are characterized by short branch lengths, which indicate small genetic distances and a recent origin and evolution. Exon 2 was divided into α-helix and β-sheet coding regions. An equal number of replacement substitutions were found within the α-helix and the β-sheet coding regions of exon 2. This finding contrasts with that of the comparisons made in other ruminant species in which far more replacement sites are found within the α-helix regions of cattle, sheep and goats (Schwaiger *et al*. 1994). It is expected that if the selective force drives the evolution of each region equally, then similar tree topologies will be reconstructed for each region (Schwaiger *et al*. 1993; Swarbrick *et al*. 1996).

In a further phylogenetic analysis, Dama alleles were aligned with ruminant DRB alleles and it was shown that all bovid alleles are monophyletic with respect to the outgroup ruminant sequences used in this analysis. The interleaving of bovid sequences suggests that the Bovidae lineages were generated prior to the divergence of bovids from a common ancestor 20 Ma (Vrba 1979). Dama alleles, although interleaved throughout the phylogeny, displayed more species-specific clustering, reminiscent of a phylogeny reconstructed with other ruminant species by Mikko *et al*. (1999). This species-specific clustering reveals that gene diversity of the Dama lineages evolved after the split between the other bovid taxa. This suggests a possible difference in the selective environment for this species, perhaps especially during the relatively recent radiation of alleles.

High allelic diversity at MHC loci is thought to establish a stronger host defence, thereby increasing individual fitness (Hughes & Nei 1989; Takahata & Nei 1990). To date there are few empirical data sets to substantiate this assumption, and many studies have shown that species having low MHC diversity are still viable (Hoelzel *et al*. 1999; Mikko *et al*. 1999). Although bontebok populations are indeed viable and show no apparent morphological signs of inbreeding depression, they are still at risk of diseases that may be transmitted by cattle existing in close proximity to the reserves. Given this possible threat of cattle disease transmission and the lack of MHC variation, bontebok may have a smaller chance of mounting immune responses against pathogens in the long term. One very important difference between the bontebok and other species with low MHC diversity is that population size of this antelope has only expanded to 2500–3000 individuals since the first bottleneck (1800s). Other species that have undergone severe population crashes such as the Northern elephant seal, American bison and African buffalo have recovered to roughly 100 000–1 000 000 animals (Mikko

et al. 1997; Wenink *et al*. 1998; Hoelzel *et al*. 1999). Although, bontebok are protected against hunting pressure, the status of the subspecies is still critical. Stochastic events (disease, drought) as well as human-mediated events (hybridization, poaching) are possible threats to the survival of the remaining populations. We recommend that the residual genetic variation unique to the bontebok be maintained through managed breeding and translocations between reserves so that DRB alleles are not lost through genetic drift over time. In addition, we suggest that DRB screening be used to monitor the genetic purity of both bontebok and blesbok herds.

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