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Polymorphisms of BoLA-DRB 3.2 gene and associated genetic relationships among four strains of Tanzania shorthorn zebu cattle

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Abstract

Bovine Lymphocyte Antigen (BoLA) genes play important roles in resistance or susceptibility of cattle to infectious diseases. The BoLA gene comprises of several loci including the most polymorphic site namely DRB 3.2. We amplified 200 DNA samples and sequenced 270 bp comprising exon 2 of BoLA-DRB 3.2 in four strains of Tanzania shorthorn zebu (TSZ) cattle (Tarime, Sukuma, Maasai, Singida white) and one breed, namely Friesian. Sequences were processed on Finch TV, aligned on Basic Local Alignment Search Tool (BLAST) online, and matched to amino acids using MEGA 6. Frequency of each allele was computed as proportion of total alleles in each population. Chi-square was used to test significance in allele frequencies. Heterozygosities were computed using Poptree 2. Putative evolutionary relationships were evaluated by Nei genetic distances. Thirty four alleles were determined, of which nine alleles are novel. The greatest number of alleles was determined in Tarime and Singida white and the lowest in Friesian. Heterozygosities were high within the animals. Phylogenetic tree showed two major clusters one for TSZ and a second for Friesian. Polymorphism at DRB 3.2 in TSZ could be one explanation for their ability to withstand various diseases and we recommend further evaluations in the breed.

Keywords: Alleles, disease tolerance, genetic variation, major histocompatibility complex, strains, Tanzania zebu

Introduction

The indigenous cattle of Africa are believed to possess inherent ability to withstand various diseases, heat stress and feed scarcity compared to crossbreds (zebu/sanga x exotic breeds) or imported exotic breeds or Bos (B.) taurus (Hansen, 2004; Maule, 1990; Mattioli *et al.*, 2000; Porter, 1991). A few examples include the West African N'Dama cattle which have been confirmed to be tolerant to trypanosomiasis (Kim *et al.*,2017) and the Kenyan Small East African zebu (SEAZ) which were shown to be resistant to Rhipicephalus (R.) appendiculatus ticks (Latif & Pegram, 1992) and to survive well in environments with poor quality forage, water scarcity and high temperatures (Western & Finch, 1986; de Clare Bronsvoort *et al.*,2013). Tanzania shorthorn zebu (TSZ) is the breed name for the indigenous cattle of Tanzania and 12 strains have been identified. The animals have been selected and bred for various purposes in different agro-ecological

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climates of the country (Msalya *et al.*, 2017; Msanga *et al.*, 2001). Tarime is one among the TSZ strains and is highly preferred by livestock keepers in northwestern Tanzania following a belief that the animals are tolerant to ticks and East Cost Fever (ECF) disease (Chenyambuga *et al.*, 2008). In a recent study, Laisser *et al.*, (2014) showed that Theileria parva (T. Parva) parasites were detected in clinically health Tarime animals indicating tolerance of the animals to the parasites. In another study, it was suggested that Tarime cattle had the ability to resist clinical development of ECF compared to another TSZ strain namely Sukuma zebu (Laisser *et al.*, 2015). Other indigenous animals such as Fipa cattle (Sanga type) were reported to be relatively high in resilience to tick-borne diseases (TBDs) compared to other cattle strains in Southern highland regions in the country (Mwakilembe *et al.*, 2007; Mwambene *et al.*, 2012a). Tarime and Fipa strains are extensively used in breeding with other strains of TSZ all over the country based on beliefs by farmers that the animals survive well in areas challenged by R. appendiculatus and T. Parva (Mwambene *et al.*, 2012a; Laisser *et al.*, 2014). Until the present time there is little scientific evidence on the levels and the mechanisms of tolerance of TSZ cattle to diseases and other stresses. To a greater extent the belief is based on the indigenous knowledge of the farmers or a few survey or observations studies conducted by local scientists (Chenyambuga *et al.*, 2008).

The bovine lymphocyte antigen (BoLA) is large cluster of tightly linked genes which play important roles in immune responses and contracting infectious diseases in cattle (Fries *et al.*, 1986). The genes are collectively known as major histocompatibility complex (MHC) in humans and other vertebrates (Edwards & Hedrick, 1998; Anderson & Davies, 1994). Association between some alleles of the BoLA genes and resistance of cattle to some of infectious disease is reported in literature (Sharif *et al.*, 1998; Lewin *et al.*, 1999; Ballingal *et al.*, 2004). The MCH is divided into three groups in literature and they are namely class I, class II and class III (Behl *et al.*, 2012). Concurrently, the bovine MHC (BoLA) which is localized to chromosome 6 of cattle genome (BTA 6) has been shown to comprise of two tightly linked loci, namely A and B (Bensaid *et al.*, 1991). Further sub-divisions of the BoLA gene into various loci including DRA, DRB, DRQ, DQA and DQB among others were reported by Groenen *et al.*(1990). Moreover, three loci of DRB (DRB 1, 2, and 3) have been researched extensively in cattle. Of these, DRB 3, was shown to be the most important locus and the polymorphisms in it have been associated to either resistance or susceptibility of cattle to various diseases compared to the other loci (Burke *et al.*, 1991). To best of our understanding, these genes have not been well studied in TSZ cattle. We therefore carried out this study to evaluate the DRB 3.2 locus in TSZ, analyze resulting polymorphisms and learn the potential ability of the TSZ to tolerate various diseases.

Lack of genetic information for TSZ and other indigenous cattle breeds of Tanzania makes it difficult to confirm the classification into existing groups. Until the present time the indigenous animals are distinguished by (i) names of ethnic groups keeping them e.g. Maasai, Sukuma, Chagga, Gogo, Mbulu, Pare, Taturu zebu and Fippa cattle; (ii) names of the locations where they are found e.g. Tarime, Mkalama Dun, and Zanzibar zebu; and (iii) names of locations and coat color e.g. Singida white and Iringa red (Msanga *et al.*, 2001). A few studies conducted previously have presented mixed results. Gwakisa *et al.*, 1994 employed random amplified polymorphic DNA (RAPD) technique and indicated that there was genetic diversity within the TSZ. In another study, Mwambene *et al.*, (2012b) used 30 microsatellite markers and showed that there was little genetic differentiation among Fipa cattle (two strains Sumbawanga and Nkasi), two TSZ (Tarime and Iringa red), as well as Ankole cattle (Sanga type). In a recent study Msalya *et al.*, (2017) used genome-wide SNP markers to genotype DNA samples from three strains of TSZ and concluded that the three TSZ strains were admixed and exhibited little genetic variations among them. It is not crystal clear whether the indigenous cattle and TSZ in particular form one breed or may be genetically distinct. Therefore the second objective of our study was to determine genetic structure of the selected strains using genotypes resulting from DRB 3.2 locus.

Materials and methods

Animal population and sampling

In total, four strains of TSZ namely Tarime, Sukuma, Singida White, and Maasai were targeted in this study. The animals are among the major strains of the TSZ breed and were sampled in four regions of Tanzania such as Mara (Tarime strain), Simiyu (Sukuma strain), Singida (Singida white strain) and Manyara (Maasai strain). The regions are distantly separated (between 300 and 800kms) and this was purposely planned to ensure that the sampled animals represented the target strain. The sampling sites and animals are shown in Fig. 1.

Although no official recording and breeding systems in existence, the animals have been classified as separate strain based on phenotypic characteristics some of which are presented in Table 1. In each strain 40 unrelated animals (mixed sex) were available for sampling, and these were obtained from different locations within the region. In addition, forty animals from the Friesian breed (sampled in a government dairy farm at Kitulo Njombe region) were also sampled to include in the study as a reference breed. From each animal, approximately 8 – 10 mls of blood were sampled from jugular vein using coated vacutainer sterile tubes with 0.5% Ethylene Diamine Tetra-Acetate (EDTA). During sampling each sample was correctly marked and temporarily stored in a cool box packed with ice blocks. The samples were sent to the laboratory at Sokoine University of Agriculture (SUA) within 24 hours after sampling. At SUA the storage condition of the blood samples was -20oC until the time of DNA purification (within 7 days).

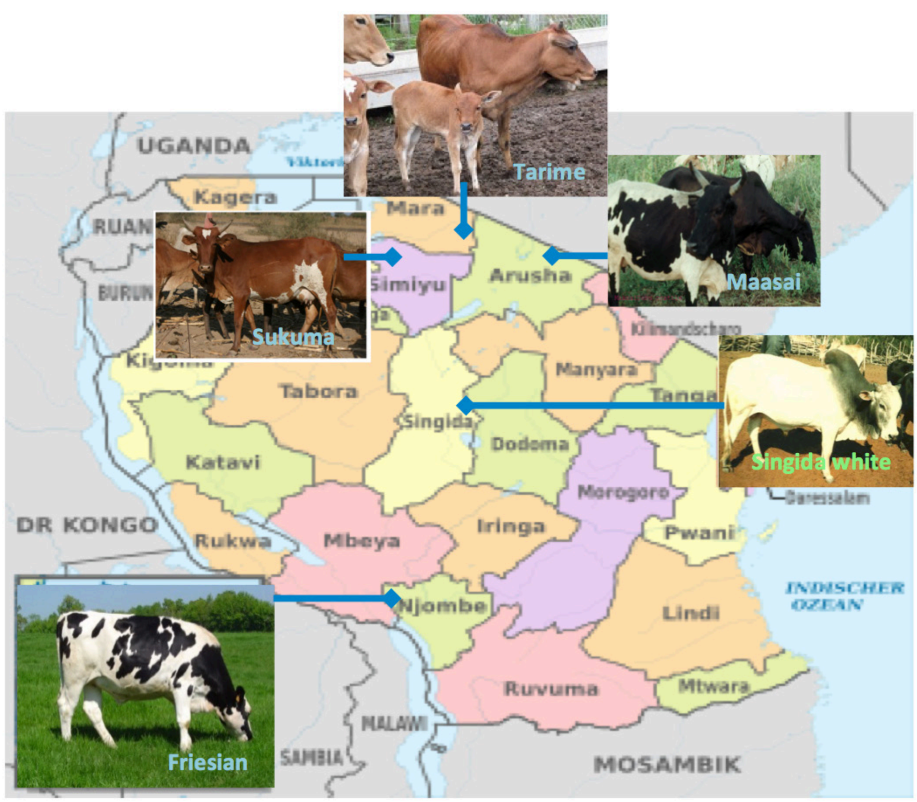

Fig. 1. A map of Tanzania showing the sampling regions (inserts: photographs representing sampled animals)

DNA purification, amplification and sequencing of DRB 3.2

DNA was extracted from the blood sampes according to instructions provided in the InvitrogenTM PureLinkTM Genomic DNA Mini Kit catalogue # K1820-02 (California, USA). The DNA was confirmed on 1.5% agarose gel and viewing on a trans-illuminator Ultra Violet (UV) radiation machine after staining with Ethidium Bromide (Fig. 2A). Concentration and purity were determined at 260 nm and 280 nm using a spectrophotometer. Amplification targeting about 270 bp in exon 2 of BoLA DRB3 (DRB 3.2) was carried out in MJ Research PTC-225 Peltier Thermal Cycler PCR in a final volume of 25 µl containing 50 ng of genomic DNA, 1 x PCR buffer, 2.5 mM MgCl2, 100 µM of dNTPs, 0.5 µM of each primer and 1 unit of Taq DNA polymerase. The PCR condition comprised of 35 cycles of 95oC (30 seconds) denaturation, 55oC (30 seconds) annealing, and 72oC (30 seconds) extension. Primers HL030 5'-ATCCTCTCTCTGCAGCACATTTCC-3' and HL031 5'-TTTAATTCGCGCTCACCTCGCCGCT-3' were previously reported by Van Eijk *et al.*, (1992). Finally we selected 150 out of 200 DNA samples (30 from each strain/breed) and these were sent to the Macrogen Laboratories Inc. in South Korea where sequencing of DRB 3.2 was performed. Sequencing was done using Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems) in an ABI PRISM 3730XL, Analyzer (96 capillary type) following their protocols. Amplified DNA samples (Fig. 2B) were used in this case.

Table 1. Some of identifiable characteristics in sampled TSZ strains

Zebu strain	Locations	Identifiable phenotypic characteristics
Maasai	Arusha and Manyara regions; migrated to other regions	The largest group among TSZ with predominant black and grey coat colors, humped, slightly large and floppy dewlap, shorter and stronger legs
Sukuma	Simiyu, Shinyanga, Mwanza, Geita and Tabora regions; migrated to other places with migrating owners	Second largest group with red and brown being as predominant colors, black and white or mixed coat colours uncommon, humped, large and floppy dewlap, short and strong legs
Singida white	Singida region (mainly in Iramba, Mkalama, and Singida rural districts) and at the boarder of Singida and Tabora region; no much migrations	Fewer in number, solid white colour, creamy white to grey, bulls are black along the head and neck, white on the rest parts of the body, humped, large and floppy dewlap, short and strong legs
Tarime	Mara region; migrated to parts of Simiyu and Mwanza regions.	Few in numbers, the predominant colour is red or fawn, medium sized sharp horns, tolerant to ECF, humped, large and floppy dewlap, short and strong legs

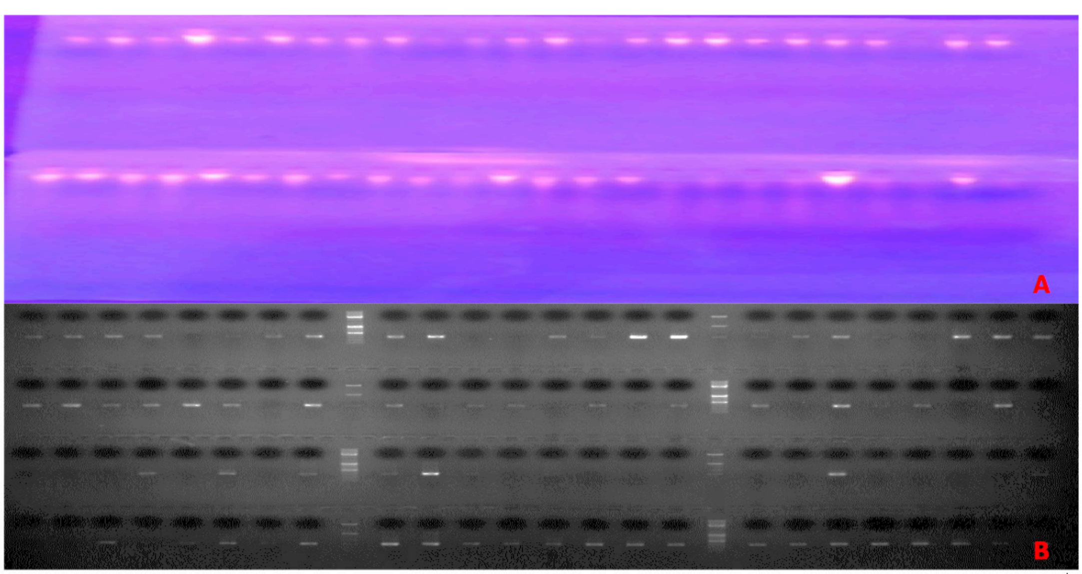

Fig. 2. Electrophoresis photographs A: purified DNA (raw); B: amplified gel-DNA

Sequences were viewed on Finch TV software and were edited manually. Briefly, miscalled nucleotides were typed and truncation of chromatogram sequences was done to obtain the required lengths (about 270bp). Mismatch and unrequired nucleotides were deleted. Then alignment with sequences from GenBank was done using the Basic Local Alignment Search Tool (BLAST) programme online. Furthermore, MEGA 6 software (Tamura *et al.*, 2013) was used to match the nucleotides to amino acid sequences. Frequencies (p) of different alleles were computed as proportion of each allele out of total alleles in each population. Chi-square was performed to test statistical significance in allele frequencies among strains. Observed heterozygosity (Ho) was obtained directly by dividing the total number of heterozygous individuals (counted heterozygous sequences) by the total number of individuals. The expected heterozygosity (He) was computed as average under the Hardy–Weinberg equilibrium in Poptree 2 software (Naoko *et al.*, 2010) using a linear formula He = $1-\sum P^{i2}$; where He = expected heterozygosity, and P_i = frequency of the i^{th} allele at a locus. Statistical significance between Ho and He in each population was also tested using Chi-Square. Putative evolutionary relationships among the study animals were evaluated using two phylogenetic (population) tree structures; a Neighbour-joining (NJ) tree of all individual alleles from nucleotide sequences of β1 domain according to Saitou *et al.*(1987), and a rooted tree constructed using unweighted pair-group method with arithmetic mean (UPGMA) based on Nei genetic distances (Nei, 1978). The MEGA 6 (Tamura *et al.*, 2013) and Poptree 2 (Naoko *et al.*, 2010) softwares were respectively used in construction of the trees and in both cases bootstrap percentage computed after 1000 replications was used to assess reliability of the trees.

Results

Alleles, allele frequencies and heterozygosities at DRB 3.2 locus in four strains of TSZ and Friesian cattle

In total 34 alleles were detected at the DRB 3 locus in our samples including nine novel alleles (DRB3.2*a – i) which are reported here for the first time. Proportionately, the novel alleles comprised 26.5% of the total alleles at this locus in our samples. A total of 17 alleles (50%) showed frequencies ≥ 5% (0.05) and were regarded as abundant. These included five novel alleles (DRB3.2*b, DRB3.2*e, DRB3.2*g, DRB3.2*i, and DRB3.2*h), about 29.4% of the abundant alleles. The DRB3.2*b was the most abundant of all alleles in the studied animal populations. Genotyped alleles and computed frequencies are presented in Table 2.

We further summarized the alleles into each of the studied population and observed a largest number of alleles in the Tarime and Singida white strains (total of 18 in each) and the lowest in the Friesian breed (Table 3). A higher number of new alleles was observed in Tarime animals compared to the rest of the indigenous strain and none on the Friesian breed. Our results revealed that the largest proportion of alleles is shared amongst the studied population and a little of these were shown to be independent in some populations. For example, there were six alleles (including three novel alleles DRB3*h, DRB3*f and DRB3*b) which were only detected in Tarime strain and the novel allele DRB3*d which was found only in Sukuma animals (Table S1).

Regarding observed (Ho) or expected (He) heterozygosities at the DRB3.2 locus, we observed a lack of statistical significance among the studied animals (Table 3). The overall range of He was between 0.972 and 0.982 among the indigenous populations (close to 1) and the value for this parameter was highest (0.997) in the Friesian breed. Looking within the indigenous population, the Tarime strain showed the greatest He (0.982).

Phylogenetic relationships among the studied animals

Concerning relationship of the animals based on the alleles, there was no clear clusters among the animals involved in the study with exception of a few of them which showed independence in some animals (Fig. 3). Closely related animals appear on neighbouring branches and share a common node on the branching point while the distantly related ones are away from each other. The length of genetic divergence (speciation) among branches ranged from 0 to 0.03. No statistical significance was observed with respect to alleles differentiation among our animals. We clarified genetic relationships following Nei's genetic distance (Table 4) and determined two major clusters; one for the indigenous cattle and a second one for the exotic Friesian cattle (Fig. 4). The indigenous animals were closely related and showed genetic distances ranging from 0.736 (in Tarime and Singida white strains) to 0.828 (between Sukuma and Singida white). All indigenous animals were farther from the Friesian breed. Among the indigenous strains, Singida white was most close to Friesian breed (Da = 0.976), while the other subtypes had the highest genetic distance (Da = 1.0). Our

Table 2. The BoLA-DRB 3.2 alleles and allele frequencies in DNA of the studied animals

Allele/population	Tarime (N=26)	Sukuma (N=16)	Maasai (N=15)	Singida white (N=29)	Friesian (N=30)	Total (N = 116)
DRB3.2*01	0.0000	0.0000	0.0333	0.0000	0.0000	0.0333
DRB3.2*02	0.0192	0.0625	0.0667	0.0345	0.0000	0.1829
DRB3.2*R-02	0.0000	0.0313	0.0000	0.0000	0.0000	0.0313
DRB3.2*04	0.0769	0.0000	0.0333	0.0172	0.0000	0.1274
DRB3.2*07	0.0192	0.0000	0.0000	0.0000	0.0000	0.0192
DRB3.2*R-08	0.0000	0.0313	0.0000	0.0000	0.0000	0.0313
DRB3.2*R-09	0.0000	0.0000	0.0000	0.0172	0.0000	0.0172
DRB3.2*16	0.0192	0.0000	0.0000	0.0000	0.0000	0.0192
DRB3.2*17	0.0385	0.0000	0.0000	0.0000	0.0000	0.0385
DRB3.2*R-19	0.0000	0.0000	0.0000	0.0000	0.1667	0.1667
DRB3.2*20	0.0385	0.0313	0.0000	0.0172	0.0000	0.0870
DRB3.2*21	0.0192	0.0313	0.0000	0.0000	0.0000	0.0505
DRB3.2*R-73	0.0000	0.0938	0.0333	0.0000	0.0000	0.1271
DRB3.2*R-141	0.0000	0.0000	0.0000	0.0172	0.0000	0.0172
DRB3.2*R-156	0.0192	0.0000	0.0000	0.0172	0.0000	0.0364
DRB3.2*R-164	0.0000	0.0000	0.0000	0.0172	0.0000	0.0172
DRB3.2*R-184	0.0192	0.0625	0.0333	0.0172	0.0000	0.1322
DRB3.2*0701	0.0577	0.0000	0.0000	0.0345	0.0000	0.0922
DRB3.2*1101	0.0000	0.0000	0.0333	0.0172	0.0000	0.0505
DRB3.2*1601	0.0385	0.0313	0.0667	0.0000	0.0000	0.1365
DRB3.2*1701	0.0000	0.0000	0.0000	0.0000	0.1667	0.1667
DRB3.2*2002	0.0000	0.0000	0.0000	0.0172	0.0000	0.0172
DRB3.2*2710	0.0000	0.0000	0.0000	0.0172	0.1667	0.1839
DRB3.2*2801	0.0000	0.0000	0.0000	0.0172	0.0000	0.0172
DRB3.2*2802	0.0000	0.0313	0.0000	0.0172	0.0000	0.0485
DRB3.2*a	0.0192	0.0000	0.0000	0.0000	0.0000	0.0192
DRB3.2*b	0.0192	0.0000	0.0333	0.1379	0.0000	0.1904
DRB3.2*c	0.0000	0.0313	0.0000	0.0000	0.0000	0.0313
DRB3.2*d	0.0192	0.0000	0.0000	0.0000	0.0000	0.0192
DRB3.2*e	0.0192	0.0000	0.0333	0.0000	0.0000	0.0525
DRB3.2*f	0.0192	0.0000	0.0000	0.0000	0.0000	0.0192
DRB3.2*g	0.0192	0.0000	0.1000	0.0172	0.0000	0.1364
DRB3.2*h	0.0192	0.0625	0.0000	0.0345	0.0000	0.1162
DRB3.2*i	0.0000	0.0000	0.0333	0.0345	0.0000	0.0678

rooted UPGMA tree (Fig. 4) clearly showed the two major clusters and small clusters within the indigenous cattle of Tanzania.

Table 3. Distribution of alleles in the strains/breeds and the observed and expected heterozygosities

Populations	N	alleles	Shared alleles	Independent alleles	Ho	He	P-value
Tarime	26	18 (7)	12 (4)	6 (3)	0.885	0.982	0.9972
Sukuma	16	11 (2)	8 (1)	3 (1)	0.875	0.973	
Maasai	15	11 (4)	10 (4)	1 (0)	0.867	0.972	
Singida white	29	18 (4)	13 (4)	5 (0)	0.862	0.972	
Friesian	3	3 (0)	1 (0)	2 (0)	0.667	0.997	

() novel alleles; Ho= observed heterozygosity; He= expected heterozygosity

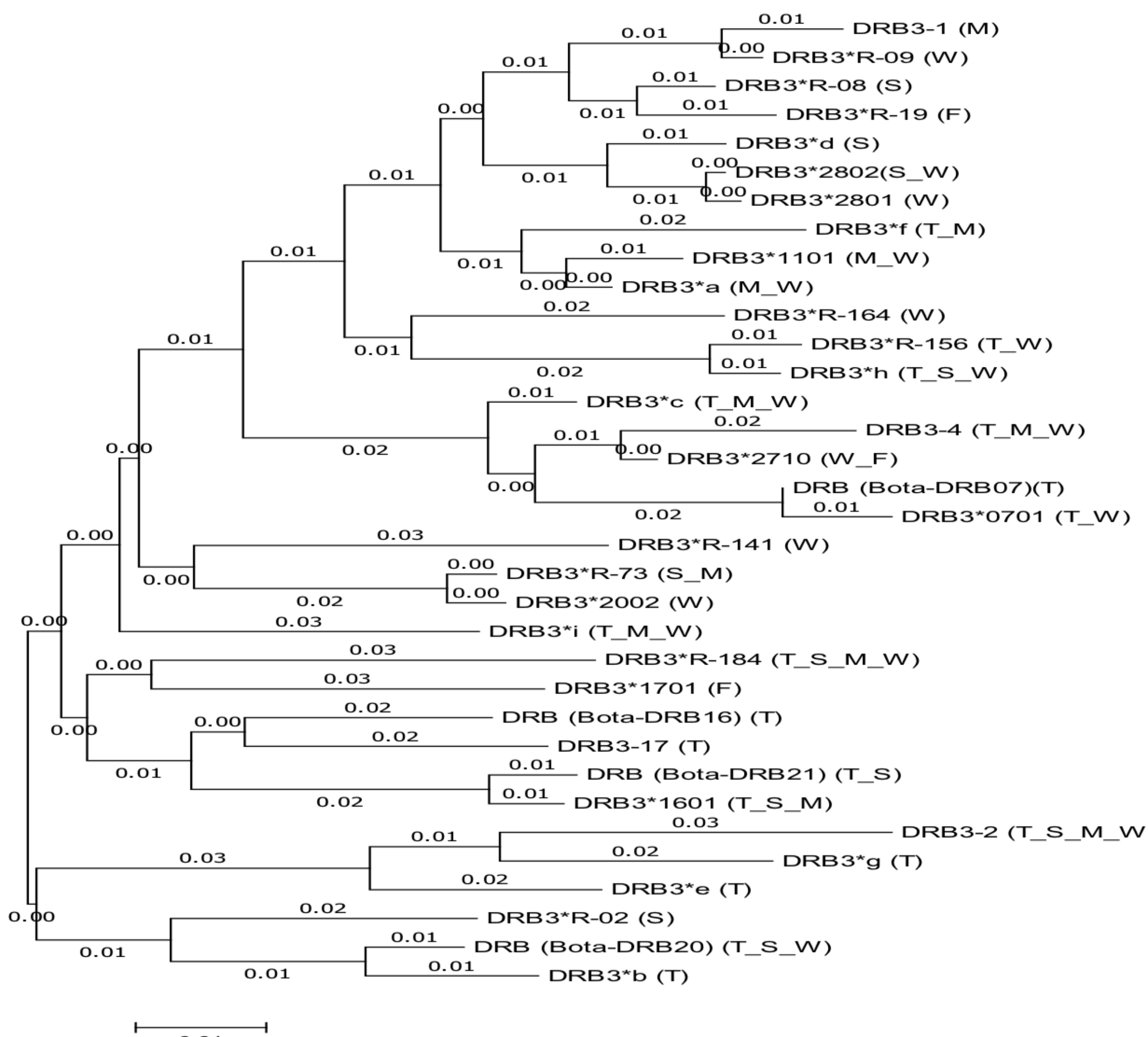

Fig. 3. Rooted UPGMA tree showing the genetic relationships among the Tanzanian cattle populations

Table S1. Alleles shared among the breeds and unique alleles to each breed

Breed	Shared alleles			Alleles unique to the breed	
Tarime	DRB3*02	DRB3-4	DRB3*20	DRB3*07	DRB3*16
	DRB3*21	DRB3*R-156	DRB3*R-184	DRB3*17	DRB3*b
	DRB3*0701	DRB3*1601	DRB3*c	DRB3*f	DRB3*h
	DRB3*e	DRB3*g	DRB3*i		
Sukuma	DRB3*e	DRB3*2	DRB3*20	DRB3*R-08	DRB3 *d
	DRB3*21	DRB3*R-02	DRB3*R-73		
	DRB3*R-184	DRB3*1601	DRB3*2802		
Maasai	DRB3-2	DRB3-4	DRB3*R-73	DRB3*01	
	DRB3*R-184	DRB3*1101	DRB3*1601		
	DRB3*a	DRB3*c	DRB3*g		
	DRB3*i				
Singida white	DRB3*2	DRB3*4	DRB3*20	DRB3*R-09	DRB3*R-141
	DRB3*R-156	DRB3*R-184	DRB3*0701	DRB3*R-164	DRB3*2002
	DRB3*1101	DRB3*2710	DRB3*2802	DRB3*2801	
	DRB3*a	DRB3*c	DRB3 *e		
	DRB3*i				
Friesian	DRB3*2710			DRB3*170	DRB3*R-19

Table 4. Nei's genetic distance matrix among the studied animals

	Sukuma	Maasai	Singida White	Friesian
Tarime	0.802	0.743	0.736	1.000
Sukuma		0.788	0.828	1.000
Maasai			0.737	1.000
Singida white				0.976

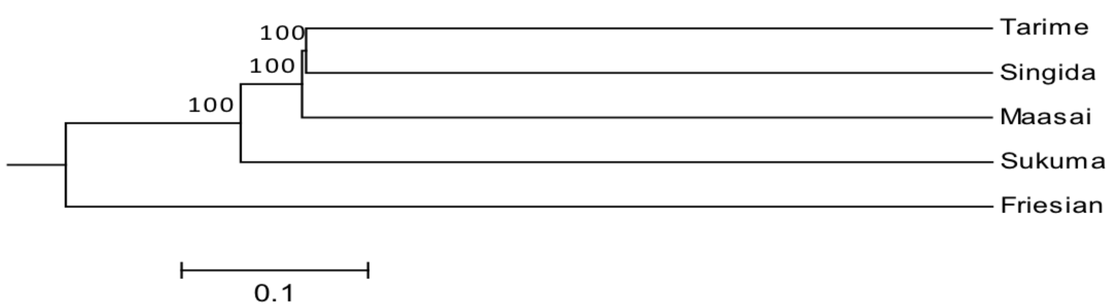

Fig. 4. Rooted UPGMA tree showing the genetic relationships among the studied populations

Discussion

DRB3-2We detected 34 polymorphic alleles at the bovine DRB 3.2 locus in four strains of TSZ cattle (the major breed in Tanzania) and a reference breed namely Friesian. The identification followed the nomenclature in Van Eijk et al., (1992). All of the detected alleles were polymorphic in our samples suggesting variation among our samples at this locus which has been shown to be highly polymorphic in other cattle breeds elsewhere (Behl et al., 2009; Nassiry et al., 2005;

Ripoli et al., 2004; Takeshima et al., 2002). It is worth noting new nine alleles which were detected in the TSZ animals some of which are strain specific at least the time we report our results. These serve as additional evidence for specific genotypes in the Bos indicus animals including TSZ possibly caused by natural section and calling for further evaluation within the breed. Recently, a study conducted by Msalya et al.(2017) showed variation in some strains of TSZ based on SNP signatures. High polymorphisms at BoLA-DRB gene has been associated with the resistance to various diseases including bovine leukaemia virus (BLV) infection (Zanotti et al., 1996) as well as dermatophilosis, cystic ovarian and mastitis among other diseases or defects (Nassiry et al., 2005). Resistance of cattle to FMD was suggested in animals with polymorphic alleles at the gene in Wanbei cattle in China (Lei et al., 2012). Resistance to diseases is a desirable and highly valuable trait in TSZ animals (Chenyambuga et al., 2008; Laisser et al., 2014; Msalya et al., 2017), however, the scientific basis for this remains speculative. Our results therefore share a great insight and calls for detailed examination among the TSZ and other local animals.

With respect to clusters shown by the phylogenic tree and separating the zebu and Friesian show the inherent differences between the groups and evidence of slowness of the most agro-pastoralists and pastoralists in rural areas to crossbreed their indigenous breeds with dairy breeds, a move that has been encouraged by the Government for a long time (Msalya et al., 2017). In Tanzania, the farmers prefer the TSZ breed to exotic breeds or TSZ x Friesian crosses because of the adaptive characteristics of the former to tolerate drought, feed shortages, poor quality forages and endemic diseases (Msalya et al., 2017). The little genetic distance among them could be a results of evolutionary or domestication history which has explained by Bradley et al., (1996).

Of interest to mention in our results are the greater values of heterozygosities in both TSZ and Friesian animals evaluated in the present study contrary to the lower values reported for some of these animals in previous studies (Gwakisa et al., 1994; Msalya et al., 2017). However, this is not surprising particularly when we consider the DRB 3.2 alone. Various researchers elsewhere have reported higher heterozygosity values in their animals. For example, Takeshima et al., (2003) reported Ho values ranging between 0.905 and 0.921 (or He between 0.887 and 0.914) in Japanese cattle breeds. In Poland, Jolanta et al.(2012) obtained He values in the range of 0.920 and 0.927. These agree well with our observations and we therefore suggest increasing heterozygosity within the studied strains or breeds, a positive factor for increased adaptation in our animals. Putative evolutionary relationships among different populations can be determined using genetic distances arising from the frequencies of alleles (Mizuki et al., 1997). We have showed that the TSZ to a large extent form one large cluster with sub-clusters. Possibly this is contributed by the separation of the animals in the country. The TSZ animals are found in different geographic locations in the country and are adapted to their local conditions differently (Chenyambuga et al., 2008). For example, the livestock keepers in Tarime, in northern Lake Victoria zone in Tanzania prefer the Tarime zebu than the Sukuma zebu on the faith that their breed is tolerant to diseases then the latter (Laisser et al., 2015). Likewise, livestock keepers preferentially keep the Ufipa strain than any other animal on such claims (Mwambene et al., 2012a). We recommend further studies particulary focusing on the possible contribution of BoLA-DRB 3.2 genotypes on the resistance or susceptibility to diseases or unfavourable climatic conditions to allow selection among the local breeds.

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