GENETIC DIVERSITY AND POPULATION STRUCTURE OF MALAWI ZEBU CATTLE

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Abstract

The aim of this study was to determine the genetic diversity and population structure of Malawi Zebu cattle using microsatellite DNA markers. Blood samples were collected in 5 administrative districts of Malawi and analysed at the DNA Laboratory, University of Malawi, Chancellor College, Zomba, Malawi. As a comparison base, samples were collected from a group of Brahman cattle performing at a commercial herd in Zomba district. A total of 15 sites were sampled giving a total of 541 random blood samples. The number of alleles per locus ranged from 6 to 15 with the minimum allele length of 98 base pairs (bp) and maximum allele length of 160bp. Malawi Zebu cattle were found to be more polymorphic than Brahman. Most of the Malawi zebu populations were not in Hardy-Weinberg equilibrium and more than 95% of the population-locus combinations were heterozygous. Gene flow among the populations was higher

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between districts than within districts, 2.04-2.40 migrants per generation between districts versus 0.36-1.27 migrants per generation within districts). The exchange of genetic material is influenced more by socioeconomic factors like trade and payment of dowry than any conscience genetic improvement practice. Three main clusters of Malawi Zebu were identified in the sampled population. With no organized conservation and improvement programmes currently available, there is need to mobilize on-farm genetic conservation and utilisation programmes and improvement strategies for the indigenous genetic resource, Malawi zebu.

Keywords: Malawi zebu, genetic diversity, microsatellite markers

Introduction

In Malawi, about 97% of the national herd of cattle comprise the Malawi Zebu which is a naturalised indigenous breed comprising several phenotypes with varied origin (Butterworth & McNitt, 1994; Jere & Msiska, 2000). Three distinct phenotypes have been reported, namely, the Angoni Zebu which has big ears, large stature and long horns and is spread in the northern region; short-horned Malawi Zebu which is small in stature and distributed in the central and southern regions of the country; and the Nkone-like Malawi Zebu owned by the Ngonis in central and northern regions (Jere & Msiska, 2000). These putative breed types have however not been supported by evidence at molecular level as to whether they are indeed distinct stocks or not, as is argued by Butterworth & McNitt (1994) that their differences are relatively insignificant. Investigation of genetic variation between populations and phenotypes is therefore required in order to develop effective conservation and utilization programmes for the Malawi Zebu, just like other livestock breeds in developing countries (Ibeagha-Awenu & Erhardt 2006; Kunene et al 2007).

In the recent past, effective breeding has emphasized on a few specialised stocks (Simianer et al 2003); however breeds that have been less studied have been at risk of population decline because of neglect of their genetic potential, lack of protection of their genetic diversity and uncontrolled crossing (Barker 2002; Mao et al 2006). Microsatellite markers have been used widely in genetic studies and pedigree verification of livestock (Ambali et al. 2000; Kumar 2004; Cervin et al 2006;

Gour 2006; Martinez 2006; Brenneman et al 2007). They are preferred markers because of their high polymorphism information content, wide spread in the eucaryotic genome, and for being amenable to cost-effective methodology of analysis (Ibeagha-Awemu & Erhardt 2005). Microsatellite typing is a good procedure for resolving breed recognitions in such livestock as goats where, due to the low level of intraspecific genetic diversity, it is difficult to differentiate breeds (Martinez et al 2006). The loci used in this study have shown unique alleles and of different distribution in the hampless breeds of cattle. Hall & Bradley (1995) and MacHugh et al. (1994) have successfully used these markers to trace the known breed histories in the European cattle breeds. This study on Malawi zebu was carried out to determine genetic diversity and population structure of the breed. Specifically the study characterised populations of Malawi Zebu cattle through analysis of genetic diversity, genetic differentiation, rate of gene flow, relatedness among the populations and potential application of the microsatellite markers in parentage tests.

Materials and methods

Sample collection

Blood was collected using sterile precisionglide needles through the jugular vein into sterile 4.5ml vacutainer tubes containing an anti-coagulant, K_3 EDTA. Samples were preserved in 70% ethanol and transported to the laboratory where they were stored in a refrigerator at 4^oC. Samples were collected from 15 sites from all the three regions of the country in 2000 (Fig 1).

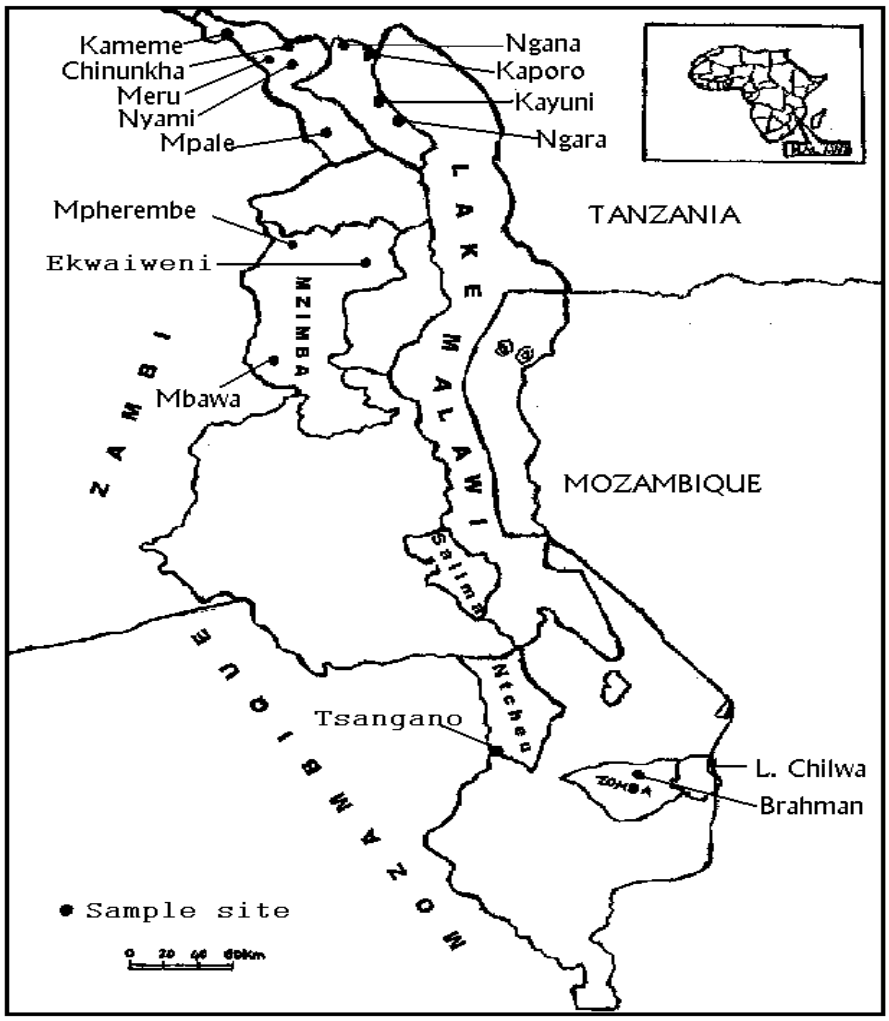


Figure 1 Map of Malawi showing sites from where cattle blood samples were collected. Brahman is a breed and was sampled from Zomba.

Two populations from Zomba were sampled as outgroups comprising a Zebu population from the eastern side of Lake Chilwa on the Mozambique/Malawi boarder and a Brahman population from a commercial farm. The Meru population was a cross of Brahman and Malawi Zebu of unknown degree of crossbreeding as no predigree records were available. The list of sampled sites is presented in Table 1. DNA was extracted using the protocol outlined by Promega Corporations for the Wizard Genomic DNA Purification System (Promega, 2000) and analysis of microsatellites was carried out following the procedure as described by Ambali et al., (2000) and visualized using silver staining on 1% polyacrylamide gel. Repeat type of each microsatellite isolate and its primers are presented in Table 2. Loci DU2S1, NOT1 and NOT3 consisted of perfect dinucleotide $(CA)_n$ repeat while RBP3, BOLA-DRBP1 and HBB consisted of imperfect repeat types $(CA)_n(TA)_n$, $(CA)_n(CT)_n$ and $(TA)_n(CA)_n$, respectively.

Table 1 Sites and districts from where the analysed cattle populations were sampled

District & site	Population number	ID*	Sample size
Chitipa district			
• Chinunkha	1		50
• Kameme	2		48
• Meru	3		13
• Mpale	4		56
• Nyami	5		46
Karonga District			
• Kaporo			
• Kayuni	6		43
• Ngana	7		36

• Ngara	8	42
	9	32
Mzimba District		
• Ekwaiweni		
• Mbawa	10	30
• Mpherembe	11	37
	12	36
Ntcheu District		
• Tsangano	13	34
Zomba District		
• Lake Chilwa		
• Brahman	14	21
	15	17

*ID = identification. In some of the tables and figures below population ID number has been used instead of site or population name.

Table 2 Microsatellite primer sequences optimised for Malawi Zebu and Brahman

Locus Code	Repeat type	Forward primer (5'-3')	Reverse primer (5'-3')
RBP3	$(CA)_n(TA)_n$	TGT ATG ATC ACC TTC TAT GCT TC	GCT TTA GGT AAT CAT CAG ATA GC
DU2S1	$(CA)_n$	GAT CAC CTT GCC ACT ATT TCC T	ACA TGA CAG CCA GCT GCT ACT
NOT1	$(CA)_n$	AGT CCA TGG GAT TGA AAG AGT TGG	CTT TTA TTC AAC AGC TAT TTA ACA AGG
NOT3	$(CA)_n$	CTG ACT ATG GTG ATA ATC CC	TCT TTT CCC TTT CCT TCC CC
BOLA-DRBP1	$(CA)_n(CT)_n$	ATG GTG CAG CAG CAA GGT GAG CA	GGG ACT CAG TCT TAT CTC TTT G
HBB	$(TA)_n(CA)_n$	GAT ATA AAA AAG ACC CAG TAG	TAC CTG AGT CAT ATG TAA TAT TCC

Data analysis

Genetic diversity variables among the populations including number of alleles per locus, total number of alleles in each population, Shannon Information Index, heterozygosity deficiency and effective number of alleles per population (Crow & Kimura 1970) were computed using POPGENE version 1.31 (Yeh et al. 1999). Observed heterozygosity, expected heterozygosity, polymorphism information content and exclusion probability were calculated using Cervus 3.0 software (Marshall et al 1998). Because the statistical distribution of alleles and heterozygosity was not known, we used mean ± 2 standard errors (s.e.) to determine whether or not the mean number of alleles and heterozygosity were statistically different between populations.

Hardy-Weinberg exact tests were carried out according to Guo & Thompson (1992) using the GENEPOP version 1.2 (Raymond & Rousset 1995). Analysis of population differentiation (F_{ST}) and genetic relatedness was carried on all the populations. DIPLOIDL program in GENEPOP was used to calculate pairwise population F_{ST} based on Weir & Cockerham (1984). The genetic distance between populations was calculated based on Nei's unbiased distance (Nei, 1978). Patterns of genetic relatedness between populations were examined using multidimensional scaling to determine the clusters in plots of the first two dimensions (Rohlf, 1992). Multilocus estimate of the effective number of migrants (Nm) using private allele method according to Slatkin (1985) was determined in the GENEPOP programme (Slatkin, 1985). Due to limited number of populations sampled in the central and southern regions of the country, the migration rates were only estimated among populations in the northern region.

Results and Discussion

Allele frequencies for the loci are provided in Table 3. Fifty-eight alleles were detected from six loci with a range of 6 to 15 alleles per locus and mean of 9.66±3.67 (sd). Of these alleles 36.2% were shared between Malawi Zebu and Brahman populations but there were no

private alleles in the Brahman population analysed at any of the loci. In loci NOT3, RBP3 and NOT1 there was at least one allele with frequency of 0.4 while in loci that showed high polymorphism, DU2S1, BOLA-RBP1 and HBB, all the alleles had frequencies of less than 0.3. This suggests that there were some alleles that were more fixed in the low polymorphic loci

alleles compared to the highly variable loci. It is also interesting to note that with the exception of RBP3 where allele 140bp and 152bp are moving towards fixation, the two highest variable loci were both of imperfect repeat type. Similar trend has been observed in Jumunapari goats where a non-perfect repeat locus, $(CT)_n(CA)_n$, had the highest variability, 10 alleles, compared to perfect-repeat loci in a battery of 23 loci of which two were non-perfect. Even the second non-perfect locus was among the third highest with six alleles (Gour et al 2006).

Measures of genetic variability at the six loci are provided in Table 4. The mean effective number of alleles was 5.17 alleles less than observed number of alleles. This suggests that there were several alleles that had low frequency which should probably be of concern in designing a conservation programme because they could easily be lost. The Shannon information index and polymorphic information content showed that the loci were highly informative suggesting high degree of polymorphism across the loci with overal means of 1.56 and 0.676, respectively. The average observed heterozygosity was more than the expected heterozygosity, 0.85 and 0.75, respectively which suggests that there was excess heterozygosity. This is further supported by the fact that there was negative heterozygosity deficiency across all the loci with mean of -0.278. High level of genetic diversity among Zebu cattle conforms to results reported in other studies. It is reported that the African Zebu developed through male-mediated introgression by crossbreeding between the Asian Zebu bulls and taurine cattle present on the continent (McHugh et al., 1997; Ibeagha-Awemu et al 2004), hence the high diversity in the Zebu breeds is believed to be due to their hybridization status (Ibeagha-Awemu & Erhardt 2006).

Table 3 Allele frequencies (Freq.) of the six microsatellite loci analyzed[1]

Microsatellite loci											
NOT3		RBP3		NOT1		DU2S1		BOLA-DRBP1		HBB	
Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq
122	0.0056	140	0.5296	102	0.4111	138	0.0213	118	0.0009	98	0.0231
124	0.0519	142	0.0009	104	0.1843	140	0.1759	120	0.0843	100	0.0361
126	0.0509	144	0.0037	108	0.0009	142	0.0056	122	0.1093	102	0.0611
128	0.4472	148	0.0287	110	0.3981	144	0.1380	124	0.1898	104	0.2037
130	0.4009	150	0.0148	114	0.0009	146	0.0269	126	0.2750	106	0.0009
132	0.0435	152	0.4102	116	0.0009	148	0.1657	128	0.0778	108	0.2731
		154	0.0120	118	0.0037	150	0.0435	130	0.0657	110	0.1815
						152	0.0944	132	0.0333	112	0.0935
						156	0.1139	134	0.0296	114	0.0028
						160	0.2148	136	0.0731	116	0.0398
								138	0.0565	118	0.0648
								140	0.0037	120	0.0093
								142	0.0009	122	0.0019
										124	0.0065
										128	0.0019

1. Underlined alleles were scored in both Malawi Zebu and Brahman populations.

Table 4 Measures genetic variability at studied microsatellite loci

Microsatellite Locus	Number of observed alleles (A)	Effective number of alleles (ne)	Shannon Index	Polymorphism information centent (PIC)	Observed heterozygosity (Ho)	Expected heterozygosity (He)	Heterozygote deficiency	Exclusion probability (EP)
RBP3	7	2.22	0.946	0.455	0.833	0.551	-0.515	0.393
DU2SI	10	6.73	2.022	0.834	0.859	0.852	-0.009	0.868
NOT1	7	2.77	1.083	0.563	0.959	0.639	-0.502	0.500
NOT3	6	2.71	1.196	0.563	0.811	0.633	-0.283	0.532
BOLA-RBP1	13	6.59	2.103	0.833	0.691	0.849	-0.186	0.876
HBB	15	5.91	2.015	0.811	0.974	0.974	-0.173	0.847

The exclusion probability ranged from 39.3% in RBP3 to 87.6% in BOLA-RBP1. The general trend observed is that loci with high level of expected heterozygosity had high exclusion probability. Similar observations have been reported in earlier studies (Cerit, et al 2004; Cervini, et al 2006). When all the loci are used together, combined exclusive probability is 99.2% which is slightly less than expected parentage level of 99.73% so that definite partenity can be decided (Cerit, et al 2004). If one were to consider the studied set of primers, loci RBP3, NOT1 and NOT3 would be replaced with high variable primers because they have got alleles that are moving towards fixation (Table 3). Alternatively, the number of loci anlaysed would be increased to reach combined probability of more than 99.73%.

Probability values for test for Hardy-Weinberg Equilibrium are presented in Table 5 and heterozygosity excess or deficiency values (F_{IS}) are presented in Table 6. Only 15.6% of the locus-population combinations were in Hardy-Weinberg equilibrium. This could be attributed to continuous mixing that is taking place between populations from several places as farmers replenish their stock or exchange their animals through dowry. In addition, the low effective population size due to deliberate choice by farmers for a specific sex or phenotype may contribute to the deviation from Hardy-Weinberg equilibrium. More than 95% of the population-locus combinations showed heterozygosity excess (Table 6). This could support the speculation that deviation from Hardy-Weinberg equilibrium may be due to population mixing hence there is Wahlund effect whereby the heterozygosity is higher than expected (Hartl & Clark, 1989).

Table 5 Probability values for test for Hardy-Weinberg Equilibrium

Population	RBP3	DU2S1	NOT1	NOT3	BOLA-RBP1	HBB
Chinunkha	0.1279	0.0000	0.0000	0.0000	0.0000	0.0000
Kameme	0.0000	0.0020	0.0000	0.0000	0.0026	0.0000
Mpale	0.0000	0.0000	0.0000	0.0000	0.0919	0.0000
Nyami	0.0269	0.1523	0.0000	0.0000	0.0000	0.0011
Meru	0.0008	0.0207	0.0060	0.0008	0.3479	0.1972

Kayuni	0.0000	0.0086	0.0000	0.0000	0.0625	0.0000
Kaporo	0.0000	0.0293	0.0000	0.0000	0.0000	0.0000
Ngana	0.0000	0.4902	0.0000	0.0000	0.0001	0.0040
Ngara	0.0293	0.0001	0.0000	0.0007	0.1865	0.0000
Ekwaiweni	0.0001	0.0002	0.0000	1.0000	0.0108	0.0146
Mpherembe	0.0004	0.0123	0.0000	0.0036	0.0000	0.0008
Mbawa	0.0000	0.0003	0.0000	0.0000	0.0004	0.0000
Tsangano	0.0000	0.0150	0.0123	0.0007	0.4702	0.0121
Chilwa	0.0154	0.1664	0.1322	0.6645	0.0555	0.0053
Brahman	0.0000	0.0015	0.0002	0.0001	0.0001	0.0132

Table 6 Heterozygosity excess and deficiency (F_{IS}) using Weir and Cockerham method. Negative values indicate heterozygosity excess while positive values indicate heterozygosity deficiency.

Population	RBP3	DU2S1	NOT1	NOT3	BOLA-RBP1	HBB
Chinunkha	-0.282	-0.541	-0.832	-0.733	0.177	-0.227
Kameme	-1.000	0.047	-0.493	-0.314	-0.196	-0.226
Mpale	-0.529	-0.445	-1.000	-1.000	-0.190	-0.254
Nyami	-0.229	0.092	-1.000	-0.876	0.303	0.328
Meru	-1.000	-0.537	-0.660	-1.000	0.087	-0.214
Kayuni	-0.658	-0.236	-0.776	-0.709	0.063	-0.492
Kaporo	-0.827	-0.339	-0.671	-0.735	0.225	-0.282
Ngana	-0.745	0.102	-0.953	-0.709	0.266	-0.337
Ngara	-0.438	-0.484	-0.939	-0.590	-0.017	-0.573

Ekwaiweni	-0.648	-0.504	-0.663	-0.036	0.115	-0.251
Mpherembe	-0.557	-0.262	-0.658	0.343	0.319	-0.151
Mbawa	-0.606	-0.288	-0.584	-1.000	-0.246	-0.419
Tsangano	-0.692	-0.343	-0.135	0.127	-0.164	-0.339
Chilwa	-0.542	-0.331	-0.337	-0.117	-0.407	-0.453
Brahman	-0.615	-0.537	-0.722	-0.889	-0.732	-0.406

The within-district-population diversity in northern Malawi is presented in (Table 7). The Chitipa and Mzimba populations had higher allelic diversity than the Karonga populations with mean±s.d observed alleles of 7.5±3.39, 7.0±3.09 and 6.17±4.11, respectively; but these were not significantly different ($p>0.05$). This is further demonstrated by Shannon's Information Index for the pooled populations where mean±s.d for Chitipa, Mzimba and Karonga were 1.46±0.51, 1.41±0.52 and 1.31±0.59, respectively.

Table 7 Within-district-population genetic variation of the Malawi Zebu populations; **na** is number of alleles per locus, **ne** is effective number of alleles and **I** is Shannon Information Index

District		Locus Code					
		RBP3	DU2S1	NOT1	NOT3	BOLA-RBP1	HBB
Chitipa[1]	na	6.00	7.00	3.00	5.00	10.00	13.00
	ne	2.16	2.81	5.43	2.55	5.25	5.94
	I	0.89	1.78	1.03	1.08	1.96	2.00
Karonga [2]	na	3.00	6.00	3.00	3.00	13.00	9.00
	ne	2.01	4.23	2.22	2.12	7.93	7.05
	I	0.73	1.58	0.87	0.82	2.23	1.61

Mzimba[3]	na	4.00	8.00	4.00	5.00	10.00	11.00
	ne	2.12	4.37	2.56	2.36	6.07	5.72
	I	0.83	1.66	1.02	1.00	2.00	1.94

1. Includes populations 1 to 5

2. Includes populations 6 to 9

3. Includes populations 10 to 12

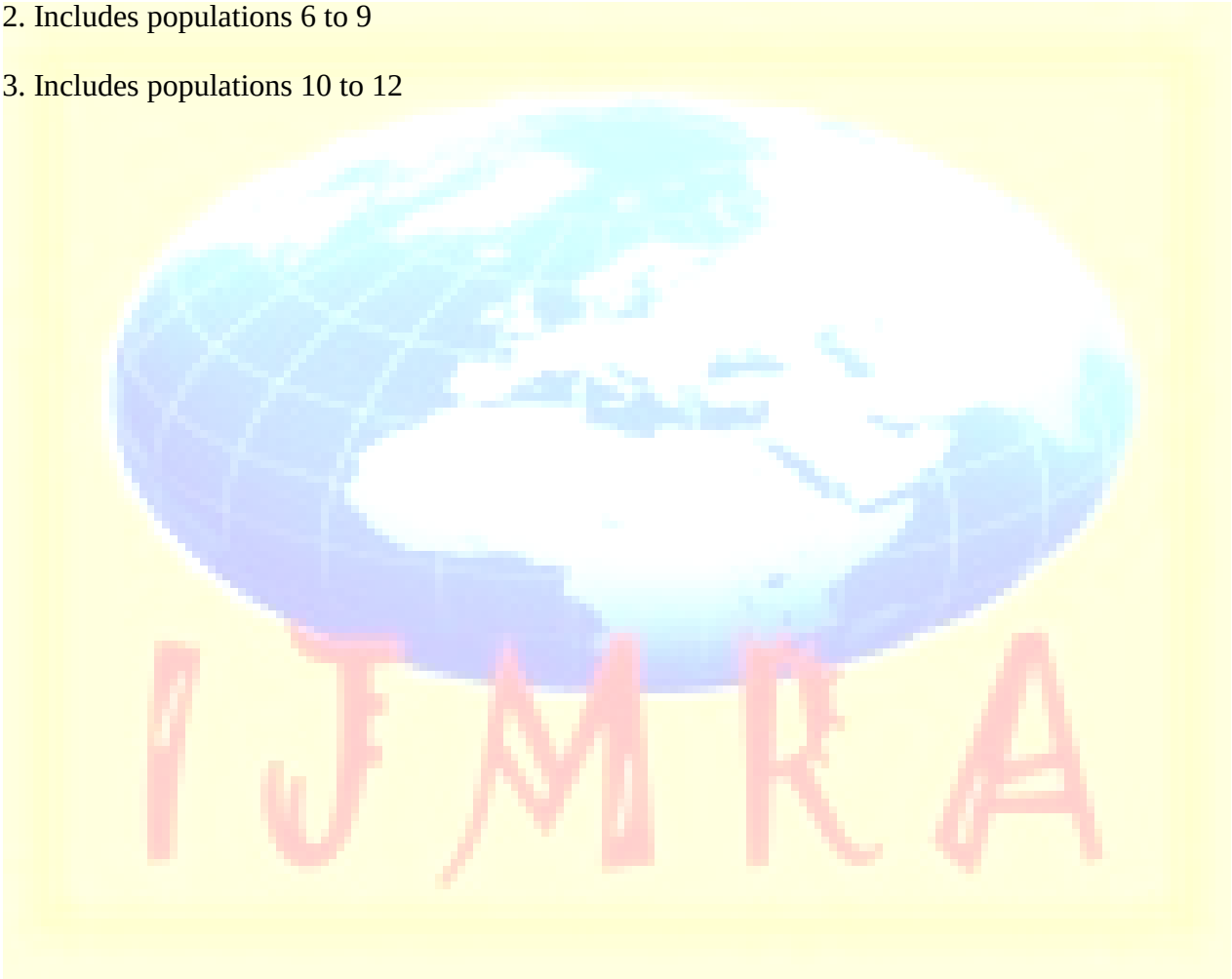


Table 8 Nei's unbiased genetic distance between cattle populations (Upper diagonal) and Genetic differentiation (F_{ST}) between cattle populations (Lower diagonal) Ekwaiw. = Ekwaiweni, Mphere.= Mpherembe, Tsang.= Tsangano, L.Chi. = Lake Chilwa, Brah. = Brahman

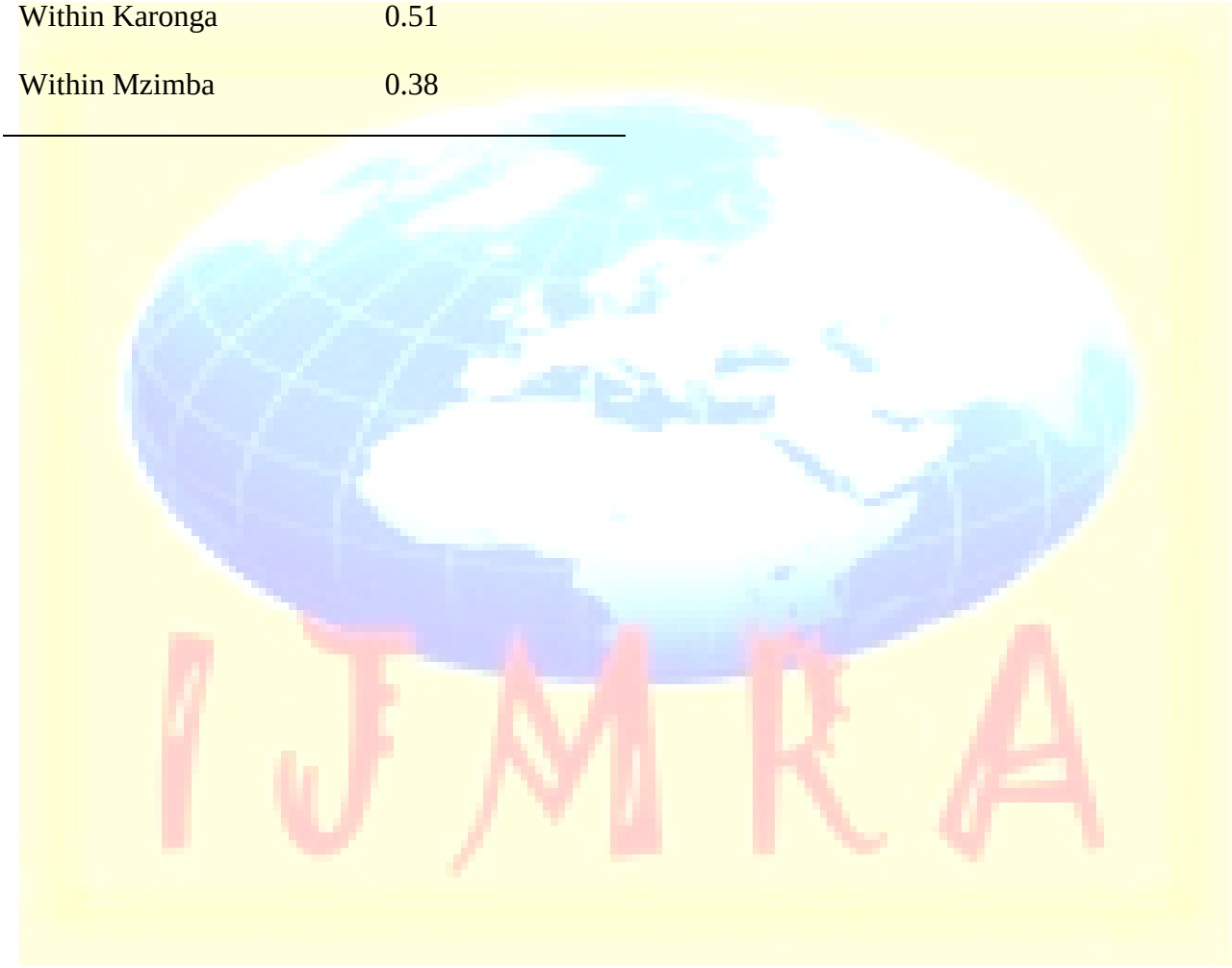
	Chinunkha	Kameme	Meru	Mpale	Nyami	Kaporo	Kayuni	Ngana	Ngara	Ekwaiweni	Mbawa	Mpherembe	Tsangano	L. Chirwa
	0.3128	0.4567	0.1650	0.2230	0.0488	0.0465	0.2254	0.0875	0.2573	0.3214	0.4790	0.4926	0.2626	0.9164
Chinunkha		0.2066	0.3545	0.3761	0.2539	0.2730	0.3813	0.3062	0.3208	0.2483	0.3237	0.5639	0.5108	0.7830
Kameme	0.112		0.3656	0.5391	0.3721	0.4566	0.6441	0.5039	0.3942	0.4245	0.5355	0.8224	0.7722	1.4256
Meru	0.169	0.077		0.2347	0.1079	0.1946	0.3026	0.1121	0.4206	0.4500	0.5313	0.7426	0.4234	1.1203
Mpale	0.087	0.147	0.172		0.2271	0.2288	0.0442	0.2739	0.4869	0.3260	0.4548	0.5376	0.3320	1.0496
Nyami	0.098	0.134	0.196	0.120		0.0339	0.2251	0.0686	0.2897	0.2753	0.4618	0.6416	0.3399	0.9221
Kaporo	0.019	0.092	0.144	0.059	0.099		0.2175	0.0755	0.2893	0.2668	0.5069	0.6260	0.3217	0.9340
Kayuni	0.018	0.102	0.174	0.102	0.102	0.011		0.2958	0.4794	0.2648	0.4268	0.5258	0.2570	0.9195
Ngana	0.099	0.136	0.220	0.147	0.018	0.098	0.098		0.3287	0.3541	0.4114	0.6464	0.3410	1.0317
Ngara	0.044	0.122	0.203	0.066	0.128	0.033	0.038	0.137		0.3141	0.7071	0.7420	0.6536	1.6187
Ekwaiw.	0.127	0.137	0.185	0.206	0.206	0.138	0.143	0.205	0.168		0.3423	0.5052	0.3168	0.9084
Mbawa	0.133	0.097	0.171	0.195	0.140	0.117	0.118	0.119	0.158	0.155		0.4015	0.3928	0.7634
Mphere.	0.177	0.120	0.196	0.217	0.177	0.171	0.188	0.170	0.175	0.259	0.146		0.3018	0.9955
Tsang.	0.174	0.170	0.244	0.255	0.192	0.203	0.207	0.189	0.226	0.258	0.185	0.158		0.6370
L. Chirwa	0.118	0.173	0.260	0.197	0.148	0.144	0.143	0.121	0.162	0.263	0.145	0.168	0.133	
Brah.	0.261	0.216	0.332	0.324	0.287	0.259	0.270	0.272	0.303	0.372	0.273	0.249	0.274	0.241

Information on population differentiation and genetic distance between populations is presented in matrices of F_{ST} and Nei's unbiased genetic distance (Table 8) while plots of multidimensional scale of F_{ST} and genetic distance are presented in Fig. 2. The high F_{ST} values suggest high degree of differentiation between populations. The two multidimensional plots show high degree of consensus whereby the Brahman population (pop. 15) was separated from the Zebu populations. Among the Malawi Zebu, the Tsangano population (pop. 13) was separated from the rest of the populations suggesting the greatest genetic distance. Although the Meru population (pop. 5) was a cross, it is closer to the Zebu than the Brahman, suggesting that there was probably a higher proportion of Zebu gene pool in the population than that of the Brahman. Three population groups of Malawi Zebu could be identified from results of this study, the Karonga-Chitipa populations which also include the Ekwaiweni population of Mzimba, the Tsangano population in Ntcheu district, and the Mbawa, Mpherembe and Lake Chilwa populations.

Table 9 presents estimates of migration rates per generation (Nm) using Slatkin's private allele method. Karonga-Chitipa-Mzimba shows the highest migration rate because many farmers in Mzimba District buy cattle from Karonga and Chitipa Districts. The animals are less expensive in the two districts than in Mzimba district due to poor cattle marketing facilities and that Chitipa farmers have large herds. Karonga-Chitipa shows a high migration rate of 2.04 per generation. This supports the claim by most farmers in Karonga that they bought cattle from Chitipa and that some farmers in Chitipa and Karonga got animals from the other district through social exchange especially dowry. Within Chitipa District, the migration rate is 1.27 per generation which is lower than the migration between Chitipa and other districts because some parts of the district have animals that trace their origins from Zambia and Tanzania. On the other hand, farmers on the southern and eastern parts of Chitipa, more especially Mpale, sell more animals to Mzimba and Karonga than to northern and western parts of the district. Within Karonga District, migration is 0.51 per generation which is lower than that of between Karonga and other districts while migration within Mzimba District, was 0.38 per generation. Among other things, this reflects the conservative within population mating system that exists within the smallholder sector where the majority of the Malawi Zebu perform. The mating systems rely on bulls and little concience exchange of breeding bulls exist.

Table 9 Estimates of number of migrants per generation (Nm)

Populations	Nm
Chitipa-Karonga-Mzimba	2.75
Chitipa-Karonga	2.04
Within Chitipa	1.27
Within Karonga	0.51
Within Mzimba	0.38



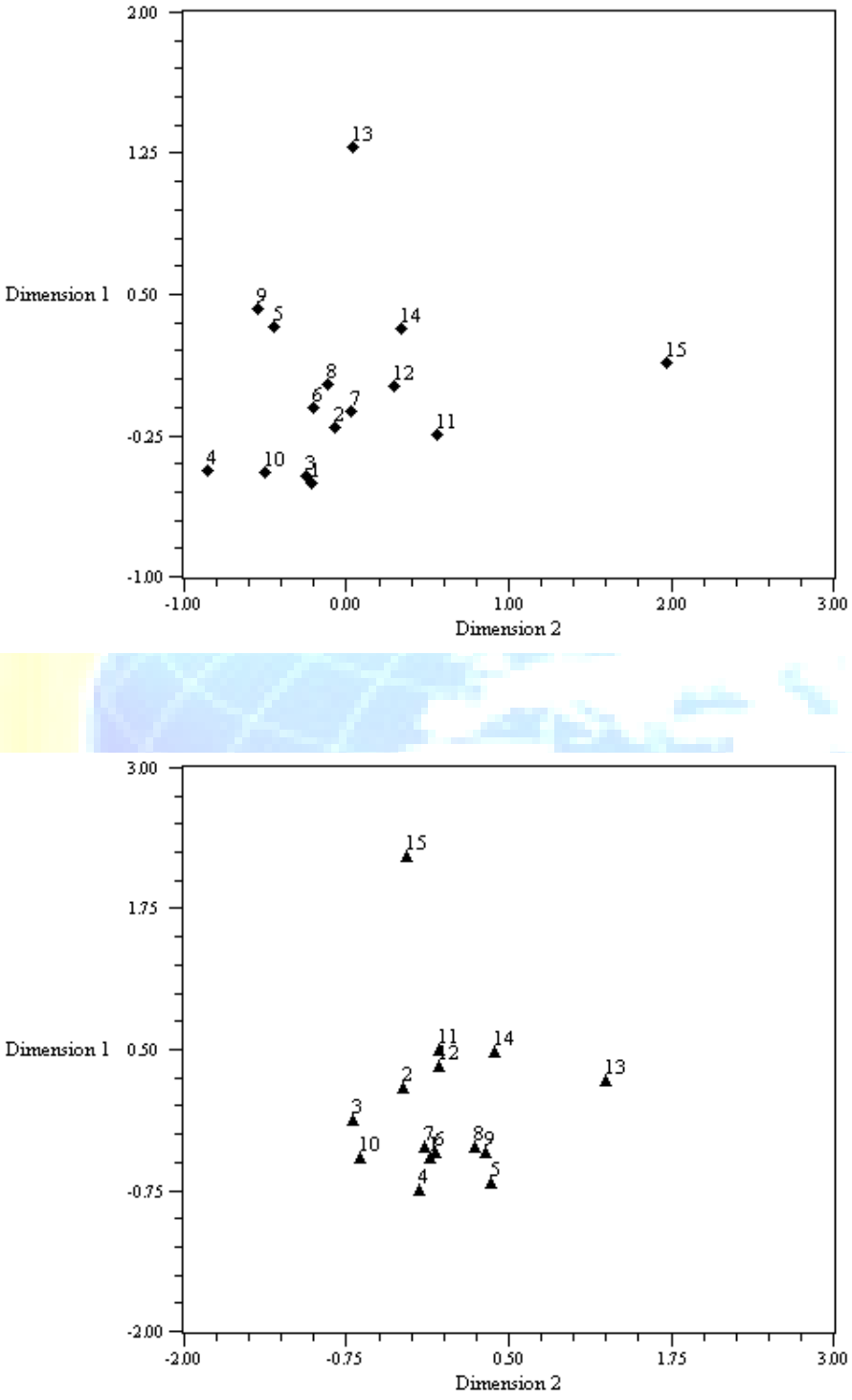


Figure 2 Plots of multidimensional scaling of F_{ST} values (top) and Unbiased Nei's distance (bottom) of cattle populations analysed. Refer to Table 1 for details of populations corresponding to number labels.

Conclusion

Although there has been some unwitting cross breeding of Malawi Zebu with exotic breeds in the past few decades, there are still several populations of unique Malawi Zebu gene pool. For example, three main crusters of Malawi Zebu were identified in the sampled population. These populations would form the critical mass for any conservation programme.

The rate of gene flow was found to be higher between than within district populations. The exchange of genetic material is influenced more by socioeconomic factors like trade and payment of dowry than any deliberate genetic improvement decision. Therefore, any conservation and improvement programmes should take cognisance of effects of movement of cattle through trade or payment of dowry and other social obligations. Besides, these movements need to be carefully monitored.

The results on within-district populations suggest that district level DNA profiling is needed to identify unique gene pools which can form the basis for genetic improvement programmes.

Acknowledgements

We are grateful to SADC/FAO/UNDP Management of Farm Animal Genetic Resources for funding this study. The encouragement of the then Chief Technical Advisor of the Project, Louise Setswaelo, is highly appreciated. We are grateful to all the farmers who allowed us to collect blood from their cattle and for the information they provided. We remember dearly our colleague and friend Lawrence Mulekano for the time we worked together and his contribution to this study, RIP.

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