November 2013 IJRSS Volume 3, Issue 4 ISSN: 2249-2496

# GENETIC PROFILING OF DAIRY CATTLE BREEDS IN SELECTED FARMS OF SOUTHERN MALAWI USING MICROSATELLITES

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## Abstract

Genetic variation has enabled selection and improvement of crop varieties and livestock breeds. In this study, the genetic variation between and within nine dairy cattle breeds was investigated at six microsatellite loci. Allelic size variation was generally high across the populations with a total of 55 alleles scored. Shannon Information Index (I) and Effective number of alleles (ne) showed that Mbala population was the least diverse (I=0.71; ne=1.9) and Mikolongwe population was the most diverse (I=1.21; ne=2.92). Only 22% of the population-locus combinations deviated from Hardy-Weinberg proportions probably due to Wahlund effect evidenced by 56 % homozygosity excess observed among the populations. The populations were significantly differentiated at 95% CI with a high $F_{ST}$ value of 15% which was supported by low gene flow (*Nm*=1.13). Cluster analysis showed that the Jersey population at Chikunda has not been greatly introgressed by Friesian alleles. Precise breeding plans and goals are required in order to control inadvertent mixing of breeds which can threaten the conservation of genetic variation within and between breeds.

**Keywords:** Dairy cattle, microsatellites, Friesian populations, Jersey populations, DNA, Genetic variation

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**Introduction**

In recent years, there has been wide interest in the study of genetic variation of farm animal genetic resources (FAnGRs) because of increasing awareness of the reduction in genetic variation caused by modern breeding strategies (Barker 2002; Mao et al., 2006). These have propagated use of few "superior" breed lines in species that have been highly selected because of emphasis on economic productivity (Simianer et al., 2003). Many countries have imported such breeds to improve productivity of their indigenous breeds through crossbreeding practices, or use of imported frozen semen of the high yielding breeds. Consequently, many indigenous breeds are being replaced in both developed and developing countries by a few high yielding breeds which to be successful require costly feed and maintenance, skilled management and benign environments. This has led to inefficient utilisation, replacement and eventual extinction of many indigenous breeds in developed and developing countries. In spite of the fact that, indigenous breeds were already well adapted to low-input/low output management systems of production and to stressful tropical environments characterised by high temperatures and high rainfall (Rege, 1995). This culminates in the loss of individual genes and gene complexes (genetic erosion) in gene pools of animal breeds, and in the reduction of genetic variation in varieties and breeds of species including cattle.

Domesticated cattle have been the subject of numerous studies of genetic variation within and between populations. Previous studies have tended to focus on European breeds of cattle using allozyme or immunoprotein techniques. The first DNA-based survey did not appear until the 1990's. Loftus *et al.*, (1994) provided molecular evidence for a predomestic divergence between zebu or humped cattle *(Bos indicus*) and taurine cattle (*Bos taurus*) using mtDNA, Displacement Loop (D-Loop) sequence distribution variation. Although other DNA based markers have been used to study genetic diversity in cattle, microsatellites have been markers of choice in genetic studies and pedigree verification in cattle and goats (MacHugh *et al*., 1997; MacHugh *et al*., 1998; Kumar et al., 2005; Martinez, 2006; Brenneman et al., 2007). They are popular because they are hypervariable, highly abundant and wide spread in the eukaryotic genome, high resolving power, low cost and easy to score (Creste et al 2004; Buhariwalla et al., 2005; Ibeagha-Awemu & Erhardt, 2005; Ibeagha-Awemu & Erhardt, 2006; Martinez et al., 2006) hence this study used microsatellites.

It was of interest to determine the genetic variation and the phylogenetic relationships of the dairy cattle breeds used in some selected farms in Malawi because the dairy industry is reported to suffer from poor record keeping (NLDMP, 1998; Jere and Msiska, 2000) hence pedigree records are not kept. It is also known that in most African cattle breeds there are many admixtures due to inadvertent mating/indiscriminate crossbreeding (MacHugh *et al*., 1997). Furthermore, the genetic variation level of the dairy cattle populations in the country had not yet been established though knowledge of the level of genetic variation would enable stakeholders in the dairy industry to make informed decisions in the development of breeding plans, goals and strategies.

## Materials and methods

### Sampling populations

A total of 327 dairy cattle were selected from nine selected large-scale farms using a multi data collection procedure. Figure 1 shows the sampling sites for the dairy farms in Blantyre and Machinga Agricultural Development Divisions (ADDs). Most of the dairy cattle in Malawi are concentrated near the main cities close to the market and processing plants in Blantyre, Lilongwe and Mzuzu, however, the majority are in the southern region (NLDMP, 1998).

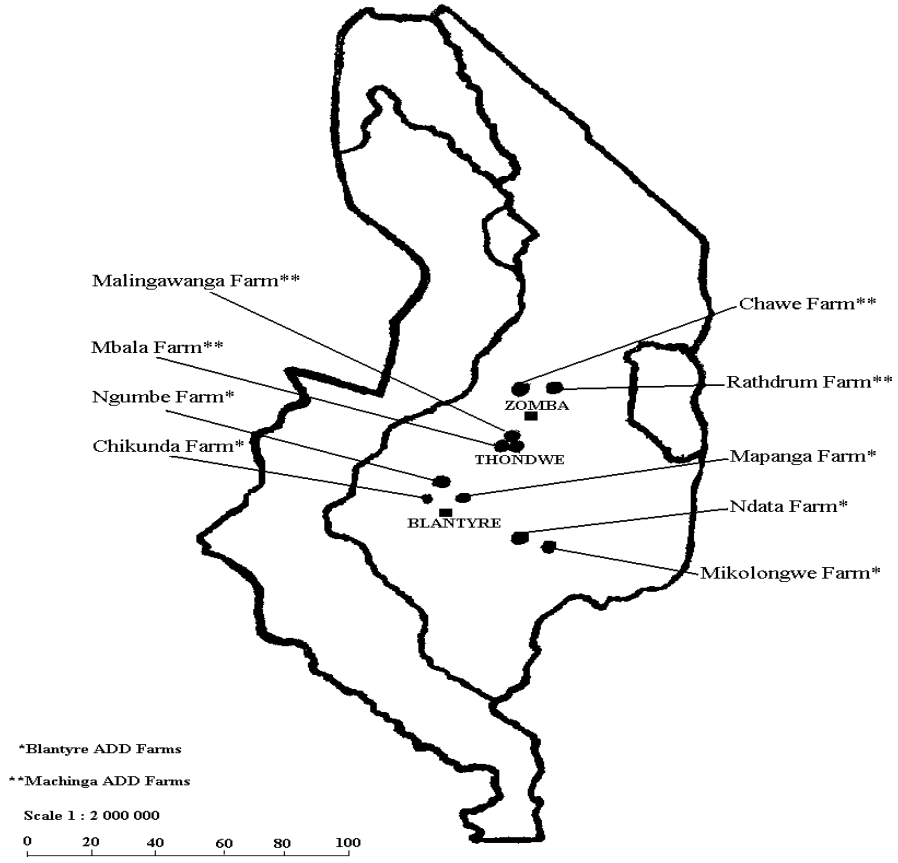


**Figure 1: Map of southern Malawi showing sampling sites of the dairy cattle farms**

## Blood Sample Collection

Whole blood samples (1-3ml) were collected from each animal, using sterile precision glide needle into the tail vein and into a sterile 4.5ml vacutainer tube blood collection sets (Becton Dickson) containing an anti-coagulant, 0.5M $K_3$EDTA (pH=8.0). Samples were preserved by adding 1ml of 95% Ethanol to each tube and transported to the Molecular Biology and Ecology Research Unit laboratory of the University of Malawi where they were stored at 4 ºC and further analysis was done. Table 1 shows the name of the farms, ADDs, breed type, and sample size per farm.

**Table 1: Population ID, ADD, population name, population code, sample size, total number of cattle on farm, % of sample and type of breed.**

| Pop ID | ADD | Population name | Pop. code | Sample size | Total # of | % of sample | Breed type |
|---|---|---|---|---|---|---|---|
| 1 | BT | Mikolongwe | MK | 40 | 43 | 93 | Malawi Zebu x Friesian |
| 2 | BT | Ndata | NDT | 24 | 250 | 10 | Holstein-Friesian crosses |
| 3 | BT | Ngumbe | NGB | 24 | 63 | 38 | Friesian crosses |
| 4 | BT | Mapanga | MPG | 24 | 160 | 15 | Friesians |
| 5 | BT | Chikunda | CKD | 14 | 38 | 37 | Jerseys |
| 6 | MHG | Chawe | CW | 11 | 13 | 85 | Jersey x Friesian |
| 7 | MHG | Rathdrum | RD | 27 | 27 | 100 | Friesian crosses |
| 8 | MHG | Mbala | MBL | 24 | 40 | 60 | Friesian crosses |
| 9 | MHG | Malingawanga | MLG | 13 | 13 | 85 | Brahman x Sussex x Friesian |

BT - Blantyre ADD

MHG - Machinga ADD

**DNA extraction**

DNA was extracted using a standard protocol outlined by Promega Corporations for Wizard Genomic DNA Purification System (Promega, 2000). Blood (300µl) in a 2.0ml microcentrifuge tube was mixed with 900µl cell lysis solution at 25ºC for 10 min followed by addition of Nucleic lysis solution (300µl). RNase enzymes (1.5µl) was added to the nucleic lysate and incubated at 37ºC for 15min, then cooled to room temperature. Protein precipitation solution (100µl) was added to the nuclear lysate, vortexed for 10-20s and centrifuged at 15000 rpm for 3 min. The supernatant was precipitated in 300µl Isopropanol in a 1.5µl tube. The precipitated DNA was separated from the suspension by centrifugation at 15000 rpm for 1 min, the supernant decanted, resulting DNA pellet rinsed in 300µl 70% ethanol at room temperature and centrifuged again at 15000 rpm for 1 min. The ethanol was decanted and the DNA air-dried for 15 min and rehydrated in 100µl DNA rehydration solution.

**DNA amplification and PCR products detection**

Microsatellite amplification reactions were done using six polymorphic microsatellite DNA markers (Table 2). The template DNA (2µl) was placed in 0.2ml PCR tube to which 10.5µl of the PCR master mix was added making a final reaction volume of 12.5µl. The PCR master mix cocktail consisted of 5.69µl PCR grade water ($ddH_2O$), 1µl of 10mM dNTP mix, 1.3 µl of 10 x PCR buffer, 1µl of 25mM magnesium chloride ($MgCl_2$), 15pmol each of both forward and reverse primers, 0.06µl of 5U *Ampli-Taq Gold*. The amplification were carried out in a Perkin Elmer GeneAmp PCR system (9600 Thermocycler version 2.01) with the following PCR conditions: *AmpliTaq-Gold* activation step at 95°C for 12 min, followed by 10 cycles of denaturation at 94°C for 30s, annealing at primer specific temperature for 15s (Table 2), and extension at 72°C for 30s. This was followed by 25 cycles of denaturation at 89°C for 30s, annealing at primer specific temperature for 15s and extension at 72°C for 15s. The final extension was at 65°C for 20min followed by a soaking temperature of 4°C.

Amplified microsatellite bands were visualized using silver sequence staining procedure on 6% polyacrylamide gels which were poured in BIORAD Sequi-Gen® GT nucleic acid electrophoresis cell. Two band size standard markers {pGem DNA marker and X174 DNA *Hinf* 1(Promega, USA)} were used to score the bands over a light box.

**Data analysis**

Genetic data was analysed using GENEPOP version 3.3(2001) (Raymond and Rousset, 1995) and POPGENE version 3.2a (Yeh *et al*., 1999) computer software packages to determine genetic diversity within and between dairy cattle populations.

GENEPOP version 3.3 (2001) was used to compute the following: test for conformity to Hardy-Weinberg Equilibrium (Haldane, 1954; Weir, 1990; Guo & Thompson, 1992), test for genotyping linkage equilibrium, test of genic differentiation, estimation of effective number of migrants using Slatikin's private allele method (Slatkin, 1985) and computation of Wrights statistics (Wright, 1969).

POPGENE Version 1.31 freeware (Yeh, et al., 1999) was employed to analyse several measures of genetic variation within and between sample populations. The following variables were computed: observed number of alleles per locus (na), effective number of alleles per locus (ne), mean number of alleles per population, mean expected number of alleles per population,

percentage polymoprphic loci (P), F-statisitcs (Wright 1978), Shannon's information index (I) (Lewontin, 1974), genetic distance and other population variance measurements such as observed heterozygosity (Ho) and expected heterozygosity(He).

**Table 2: Microsatellite loci used to assay genetic variation in the dairy cattle surveyed**

| Locus code & gene | Chromosome {synthetic group} | Amplification primers 5'-3' | | | | | | | | Repeat motif | $T_{ann.}$ (°C) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DU2S1 | | GAT ACA | CAC TGA | CTT CAG | GCC CCA | ACT GCT | ATT GCT | TCC ACT | T | (CA)n | 60°C |
| HBB -globin locus | 15 {U19} | GAT TAC | ATA CTG | AAA AGT | AAG CAT | AA G ATG | ACC TAA | CAG TAT | TAG TCC | $(TA)_2(CA)_{17}$ | 55°C |
| RBP3 Retinol-binding protein 3 gene | 28 {U29} | TGT GCT | ATG TTA | ATC GGT | ACC AAT | TTC CAT | TAT CAG | GCT ATA | TC GC | $(CA)_8(TA)_4$ | 55°C |
| BOLA-DRBP1 MHC class II DRB pseudogene | 23 {U20} | ATG GGG | GTG ACT | CAG CAG | CAG TCT | CAA CTC | GGT TAT | GAG CTC | CA TTT | $(GT)_{18}(GA)_8$ | 55°C |
| NOT2 | | GGT GGA | GCT GTC | GTT ATA | ATC CAC | TAG AAC | AAT TGA | TTG GC | | (CA)n | 55°C |
| BOLA-DR2B MHC class II DRB2 gene | 23 {U20) | AGG TCC | CAG AAC | CGC ACT | CGA CAC | GGT CTG | GAG GAC | CGA GTA | GC | $(GT)_5(N)_{20}(GT)_4(GA)_5$ | 55°C |

n- number of repeats

NTSYSpc version 2.11c (Rolhf, 2001) used Nei's unbiased genetic distances matrices for the populations generated by POPGENE Version 1.31 to construct dendrograms from the Sequential Agglomerative Hierarchical and Nested (SAHN) clustering method using the Unweighted Pair-Group Method with Arithmetical averages (UPGMA) algorithm (Sneath & Sokal, 1973). The dendrogram showed the genetic relationships among the dairy cattle populations.

**Results and Discussion**

**Allelic variations**

A total of 55 alleles were scored at six microsatellite loci in 327 dairy cattle. Allelic size variation among the nine dairy cattle populations was generally high across all the six-microsatellite loci. (Table 3).

**Table 3: Six microsatellite loci, number of alleles/locus, allele size range**

| Locus | # of alleles/locus | Allele size range |
|---|---|---|
| DU2S1 | 11 | 138-158 |
| HBB | 10 | 96-120 |
| RBP3 | 5 | 140-154 |
| BoLADRBP1 | 14 | 120-146 |
| NOT2 | 6 | 80-98 |
| BoLADR2B | 9 | 132-162 |

The mean observed number of alleles (na) per population at six loci was higher than the mean effective number of alleles (ne) at all the population-locus combinations since observe number number of alleles estimates include rare alleles (Figure 2) (Kamonrat, 1996). Consequently, a large difference between (na) and (ne) in this study indicates presence of considerable amount of low frequency alleles (rare alleles) among the populations which essentially differentiate them.

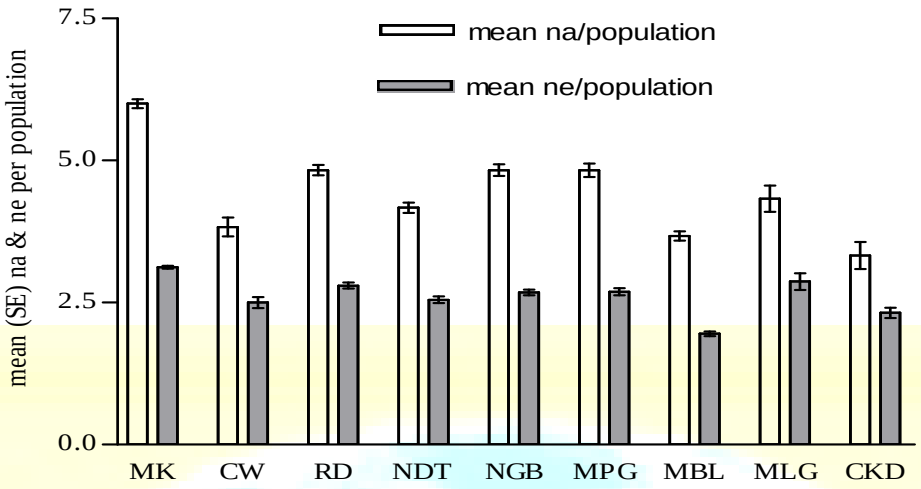


Figure 2: Mean and standard error (SE) observed (na) and expected (ne) of alleles per individual population.

Mikolongwe (MK), Chawe (CW), Rathdrum (RD), Ndata (NDT), Ngumbe (NGB), Mapanga (MPG), Mbala (MBL), Malingawanga (MLG), Chikunda (CKD

Mean Shannon information Index (I) ranged from 0.71 at Mbala to 1.21 at Mikolongwe and the mean number of alleles per population varied from 3.33 in the Jersey-Chikunda population to 5.33 in the Malawi Zebu x Friesian crossbreds-Mikolongwe population (Table 4). Both effective number of alleles (ne) and Shannon Information Index (I) as measures of genetic diversity confirmed Mbala population as the least diverse and Mikolongwe population as the most diverse (Table 4). In terms of mean observed number of alleles (na), Chikunda was the least diverse population. The mean observed number of alleles findings of this study were comparable to 3.4 (n=34) and 3.1 (n=20) of the Jersey population at Channel Islands using 20 microsatellite loci (MacHugh *et al.*, 1997). Moazami-Goudarzi, *et al.*, (1997) using 17 microsatellite loci on 13 different cattle breeds also found that the Jersey displayed the least mean number of alleles, 6.0 (0.6) (n=50), but was higher than that found in the two studies cited above. The low genetic variation observed in the Jersey breed could be attributed to small population sizes, (founder effect) compounded by genetic isolation. The Jersey breed originated from the small Channel Islands of England and it has effectively existed in isolation because of strict breeding practices for about 200 years (MacHugh *et al.*, (1997). Consequently, inbreeding could have caused the reduction in genetic variation with loss of some alleles in the Jersey breed.

Overall the mean number of alleles did not vary much among the Friesian populations at Ndata, Mapanga, Ngumbe, Rathdrum, Mbala and Malingawanga which displayed 3.83, 4.67, 4.50, 4.67, 3.67, and 4.17, respectively and 5.33 alleles were detected at Mikolongwe registering the highest among all the dairy cattle populations (Table 4). MacHugh *et al.*, (1997) and Moazami-Goudarzi, *et al.*, (1997) in their studies found 4.8(n=40) for the Holstein-Friesians in the Netherlands and 6.76 (n=50) in the Holsteins in the other study, respectively. The latter was markedly higher for Holstein-Friesians, whereas the former compares very well with the mean number of alleles exhibited in this study. The high mean number of alleles displayed by the Malawi Zebu x Friesian at Mikolongwe was probably due to input of both breeds making up the cross.

**Allelic variation: Heterozygosities**

The observed (Ho) and expected (He) heterozygosities per population are presented in Table 4. The mean observed heterozygosity (Ho) ranged from 0.41 in the Friesian crosses at Mbala to 0.64 in the Jersey x Friesians at Chawe. The Malawi Zebu x Friesians at Mikolongwe displayed the highest mean observed heterozygosity, 0.62 among Friesian populations (Table 4).

The overall pattern for heterozygosity was similar to that of mean number of alleles especially among the Friesian populations. The Friesian populations observed heterozygosities (Ho = 0.41 - 0.62), were comparable to those (Ho = 0.43 - 0.48) observed among five Holstein-Friesians surveyed by Hanslik *et al.*, (2000). Hanslik *et al.*, (2000) using 39 microsatellite loci on the New World Holstein-Friesians i.e Canadian HF, American HF, Danish HF and German HF, found Ho = 0.39, 0.39, 0.35 and 0.30 respectively which were much lower than the values found in this study. In contrast, in same study, Hanslik *et al.*, (2000) observed Ho = 0.46 (n=15) in original Friesian samples from England with no introgression from the New World Holstein-Friesians. MacHugh *et al.*, (1997) in another study, using 20 microsatellite loci on Holstein-Friesians from Netherlands found Ho = 0.55 which was higher than the figures in the other studies. Apparently, the level of heterozygosites are moderate and do not vary much among the different studies. Interestingly, both Jersey populations at Chikunda and Chawe exhibited high heterozygosities, 0.51 and 0.64, respectively. The figures for the Jerseys were markedly higher than 0.44 for the Jersey at Channel Islands (MacHugh *et al.*, 1997), but was comparable to Ho = 0.53 observed by

**Table 4: A summary of mean observed number of allele (na), mean effective number of alleles (ne), mean observed heterozygosity, mean expected heterozygosity and Shannon Information Index (I) at six loci**

| Dairy cattle populations | Type of breed | ADD | N | Mean na/population (SD) | Mean ne/population (SD) | Mean Ho/population (SD) | Mean He/population (SD) | Mean Shannon Information Index |
|---|---|---|---|---|---|---|---|---|
| **Jersey populations** | | | | | | | | |
| 1. Chawe | Jersey x Friesian | Zomba | 11 | 3.67 (1.97) | 2.53 (1.14) | 0.64 (0.36) | 0.53 (0.30) | 0.95 |
| 2. Chikunda | Jersey | Blantyre | 14 | 3.33 (2.07) | 2.32 (1.25) | 0.51 (0.41) | 0.44 (0.36) | 0.80 |
| **Friesian Populations** | | | | | | | | |
| 3. Mikolongwe | Malawi Zebu x Friesian | Blantyre | 40 | 5.33 (2.80) | 2.92 (0.93) | 0.62 (0.15) | 0.63 (0.13) | 1.21 |
| 4. Ndata | Holstein-Friesian | Blantyre | 24 | 3.83 (1.83) | 2.39 (1.10) | 0.47 (0.21) | 0.53 (0.19) | 0.95 |
| 5. Ngumbe | Friesian crosses | Blantyre | 24 | 4.50 (2.43) | 2.65 (1.20) | 0.58 (0.11) | 0.57 (0.18) | 1.06 |
| 6. Rathdrum | Friesian crosses | Zomba | 27 | 4.67 (2.34) | 2.64 (1.33) | 0.49 (0.15) | 0.54 (0.24) | 1.05 |
| 7. Mbala | Friesian crosses | Zomba | 24 | 3.67(1.97) | 1.90 (0.93) | 0.41 (0.32) | 0.38 (0.27) | 0.71 |
| 8. Mapanga | Friesians | Blantyre | 24 | 4.67 (2.66) | 2.65 (1.40) | 0.53 (0.18) | 0.54 (0.22) | 1.02 |
| 9. Malingawanga | BrahmanxFriesianxSussex | Zomba | 13 | 4.17 (2.64) | 2.85 (1.87) | 0.53 (0.25) | 0.55 (0.25) | 1.02 |

ADD=Agricultural Development Division

Moazami-Goudarzi, *et al.*, (1997). A high heterozygosity level should be expected at Chawe because it has not been purebred, so it would not breed true to its type, because it should be an admixture of Friesian and Jersey breeds. In contrast, the Jersey population at Chikunda was only two years old at the time of the study having only recently replaced the whole Friesian herd that was on the farm since 1999. The breed was imported from Zimbabwe and they have only used imported frozen semen.

The Malawi Zebu x Friesian at Mikolongwe recorded the highest mean observed number of alleles, 5.33 (2.80) and high heterozygosity, Ho = 0.62 possibly be due to the introgression of African Zebu alleles into the taurine breed. MacHugh *et al.*, (1997) recorded the highest mean observed number of alleles of seven, in five pooled African Zebu populations, (n=200). African Zebus have been influenced by historical zebu-taurine crossbreeding, hence their high allelic diversity evident from high mean number of alleles and high heterozygosities (MacHugh *et al.*, 1997). For example, the Maure breed, an African Zebu from Senegal recorded the highest mean number of alleles equal to 6.2 (n=55) and 5.3 (n=20) compared to European and African taurine and Asian Zebus in the same survey, whilst the pooled African Zebus from Northern and Southern Sudan, Senegal, Mauritania and Nigeria (n=200) displayed Ho = 0.63 compared to Ho = 0.57 displayed by pooled Asian Zebus (n=33) from northern India and Pakistan (MacHugh *et al.*, 1997). Hence, the African Zebus apparently exhibit the highest genetic variation, than European taurine, African taurine, and Asian Zebus. High allelic diversity observed in the zebu-taurine crossbreds was reported to be undoubtedly an artefact of admixture and the consequent input of both taurine and Zebu alleles (MacHugh *et al.*, 1997).

**Conformity to Hardy-Weinberg Equilibrium (HWE) and test for linkage disequilibrium**

Results for the Hardy-Weinberg Equilibrium exact test are presented in Table 5. Twenty two percent of the population-locus combinations deviated from HWE. Generally, the population-locus combinations that deviated from HWE furthermore exhibited heterozygosity deficiency or were monomorphic (Table 5) Deviations from expected HWE genotypes' proportions can be caused by the presence of overdominant selection, presence of null alleles leading to a false observation of excess homozygotes, inbreeding within populations, and the presence of population substructure leading to Wahlund effects (MacHugh, 1996; Devlin *et al.*, cited by

Ambali, 1996). These forces are more pronounced in populations, which have been subjected to human interventions than in wild populations (Ambali, 1996). Deviations from HWE were expected because dairy cattle are domesticated animals that have been greatly influenced by artificial selection and the major dairy breeds, Holstein-Friesians and Jerseys have undergone intense selection. Furthermore, most of these herds usually have small founding populations since a few individuals, unrepresentative of original gene pool would form a founding stock in most of the dairy farms. In addition, most of the dairy populations are sub-divided and would experience Wahlund effects in which inbreeding and random genetic drift occuring in the sub-divided populations increases genetic differentiation among populations. Wahlund effect is further evidenced by 56% homozygosity excess observed among the populations (Table 5)

With the exception of DU2S1 and BoLADRBP1 locus pair, all the other loci pairs showed no significant linkage disequilibrium at $p<0.05$ (Data not shown). This implies that the two loci were not as informative during the genetic analysis since they were assorting independently. Linked loci experience similar evolutionary processes and will therefore not provide independent estimates of heterozygosity and measures of population subdivision. Linked loci could actually be physically strongly linked on the same chromosome (Augustin, 1999). However, MacHugh, (1994) using the same loci on European and Continental taurine as well as African Zebu cattle breeds did not observe any linkage disequilibrium between these loci. In addition, microsatellite loci BoLADRBP1 and BoLADR2B are physically linked on chromosome 23 together with PRL locus but these were in linkage equilibrium in this study (MacHugh, 1996).

**Table 5: Hardy Weinberg test: Heterozygosity excess and deficiency ($F_{IS}$ estimates) and estimates of exact P-Values using Markov chain method at six loci ($P \leq 0.05$).**

| Population | DU2S1 | | HBB | | RBP3 | | BoLADRBP | | NOT2 | | BoLADR2B | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $F_{IS}$ | *P*-value | $F_{IS}$ | *P*-value | $F_{IS}$ | *P*-valu | $F_{IS}$ | *P*-valu | $F_{IS}$ | *P*-value | $F_{IS}$ | *P*-valu |
| Mikolongwe | +0.0 | 0.38 | +0.1 | 0.04 | +0.40 | 0.01 | -0.08 | 0.10 | -0.19 | 0.98 | -0.35 | 1.00 |
| Chawe | -0.36 | 0.94 | -0.32 | 1.00 | -0.250 | 1.00 | +0.0 | 0.28 | - | 0.00 | -0.26 | 1.00 |
| Rathdrum | -0.22 | 1.00 | +0.1 | 0.02 | -0.086 | 1.00 | +0.2 | 0.02 | +0.2 | 0.04 | -0.29 | 1.00 |
| Ndata | +0.3 | 0.03 | -0.01 | 0.07 | +0.44 | 0.04 | -0.05 | 0.05 | +0.0 | 0.60 | -0.18 | 1.00 |
| Ngumbe | +0.2 | 0.15 | +0.0 | 0.09 | -0.255 | 1.00 | +0.0 | 0.45 | -0.01 | 0.65 | -0.39 | 1.00 |
| Mapanga | +0.2 | 0.01 | -0.15 | 0.05 | -0.235 | 1.00 | +0.3 | 0.00 | +0.1 | 0.40 | -0.52 | 1.00 |
| Mbala | -0.03 | 0.09 | -0.14 | 0.86 | - | 0.00 | -0.15 | 0.91 | +0.1 | 0.33 | -0.10 | 1.00 |
| Malingawanga | +0.2 | 0.14 | -0.09 | 0.75 | +0.25 | 0.07 | -0.06 | 0.85 | +0.0 | 0.60 | -0.04 | 1.00 |
| Chikunda | 0 -0.22 | 0.84 | -0.25 | 1.00 | 0 - | 0.00 | -0.00 | 0.33 | 1 - | 0.00 | -0.28 | 0.99 |

- Dashes represent monomorphic loci.

Negative $F_{IS}$ values indicate heterozygosity excess while positive values indicate heterozygosity deficiency, 56% population-locus combinations show heterozygosity excess, 44% heterozygosity deficiency.

**Population differentiation**

Genic differentiation using Fisher' exact probability test showed that the dairy cattle populations in the study were highly and significantly differentiated across all loci ($p \leq 0.05$) (Data not shown). The summary of F-Statistics for all loci is presented in Table 6. An inbreeding coefficient, $F_{IS}$ value of –0.03 (3%), (Table 6) was obtained for all loci across the dairy cattle populations surveyed in this study indicating an overall low level of inbreeding within the populations which is normal for dairy cattle since inbreeding within the population is not common practice. The mean $F_{ST}$ value across all populations was 0.15 (15%) (Table 6), indicating relatively high genetic differentiation among the dairy cattle populations that could be ascribed to differences between allele frequencies. Population differentiations of more than 15% are considered great rather than moderate (Hart & Clark 1997; Dorak 2002) and are associated with low gene flow among the populations. Gene flow in this study was rather low (Nm=1.13; Table 6) which is consistent with general practice of restricting movement of dairy cattle

therefore limiting mating with close herds resulting in low gene flow. The overall reduction in heterozygosity of an individual relative to the total population ($F_{IT}$) was 18%.

**Table 6: Summary of F-Statistics and gene flow for all loci at six microsatellites**

| Locus | $F_{IS}$ | $F_{ST}$ | $F_{IT}$ | N*m** |
|---|---|---|---|---|
| DU2S1 | 0.00 | 0.13 | 0.12 | 1.75 |
| HBB | - 0.09 | 0.09 | 0.16 | 1.30 |
| RBP3 | 0.10 | 0.46 | 0.41 | 0.37 |
| BoLA-RBP1 | 0.02 | 0.13 | 0.11 | 1.98 |
| NOT2 | 0.02 | 0.15 | 0.13 | 1.67 |
| BoLA-DR2B | - 0.33 | - 0.09 | 0.18 | 1.17 |
| **All** | **- 0.03** | **0.15** | **0.18** | **1.13** |

*N*m* = Gene flow estimated from $F_{ST} = 0.25 (1- F_{ST})/F_{ST}$

**Phylogenetic relationships**

The UPGMA dendrogram in (Figure 3) shows the genetic relationships among the sampled dairy cattle based on Nei's (1978) genetic distances. It shows four clusters, the Malawi Zebu x Friesian crossbreds, the Friesian populations from Rathdrum, Chawe, Malingawanga Mbala and Mapanga, the Friesians from Ndata and Ngumbe and the fourth cluster of the Jerseys at Chikunda.

The dendrogram for the nine dairy cattle populations was rather distorted because some of the dairy cattle are hybrid populations hence are not discrete evolutionary lineages. Felsenstein, (1982) and Nei (1987) cited by MacHugh *et al.,* (1997) argue that such phylogenetic trees with admixtured populations violate the fundamental principles of phylogeny reconstruction. The populations at Mbala and Mbala were clustered the closest, implying that these two populations are the most genetically closely related Friesians. Generally the Friesians in cluster two are closer genetically.

The Malawi Zebu x Friesian crosses at Mikolongwe were genetically closer to the Friesians in cluster two signifying more introgression of Friesian alleles. This is consistent with the current

breeding policy of using Friesian semen, and confirms the fact that the dairy herd at Mikolongwe as a distribution farm, has been upgraded more to the Friesian breed.

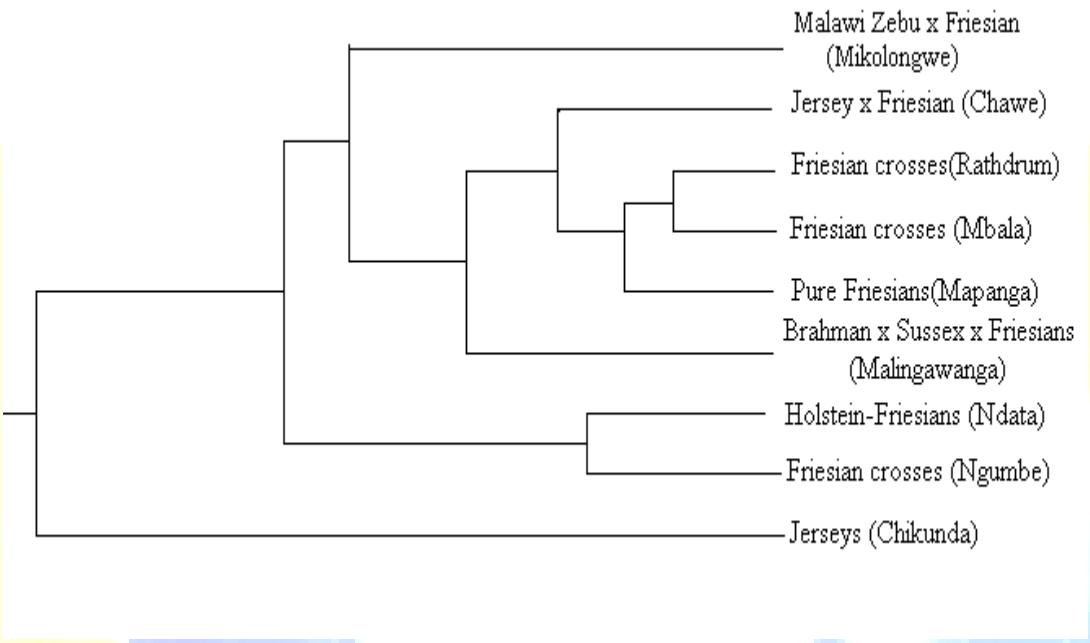


Figure 3: UPGMA dendrogram showing the genetic relationships among the sampled dairy cattle based on Nei's genetic distance

The Holstein-Friesians at Ndata have clustered together with the Friesian crosses at Ngumbe although these are geographically distant This suggests that the Friesian crosses at Ngumbe have a higher introgression of Holstein-Friesian genes.

The Jerseys at Chikunda has been distinctively isolated from the Friesian populations signifying that it is genetically very distant from the Friesian populations because it is a different breed that has been kept discrete.

**Conclusions**

This study has shown that there is considerable genetic variation among the dairy cattle populations and it has also confirmed genetic distinctiveness of Jerseys at Chikunda. The genetic variation of the dairy breeds in the country is comparable to that of the British Isles and Continental European cattle, which is low, compared to that of indigenous African Zebu cattle.

The study has also shown that crossbreds of European taurine with African Zebus, like the population at Mikolongwe results in higher genetic variation than the distinct European Friesians and Holstein-Friesians at Mapanga and Ndata, respectively.

Inbreeding among the populations is low but it needs to be checked because as have been observed, the populations can be fixed for two alleles or become monomorphic at some of the loci. This seems to be particularly the case with Mbala, hence it having the least within-population genetic variation.

**Acknowledgement**

The study was made possible by financial assistance from USAID. The research group is indebted to farm owners, dairy farm managers and staff for permission to collect blood samples and information about the herds. We are grateful to Mr Gerald Mwakifuna (Zomba veterinary officer) and Mr Austin Ngwira (Lilongwe, Land O' Lakes official) for technical information and assistance in collecting cattle blood.

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