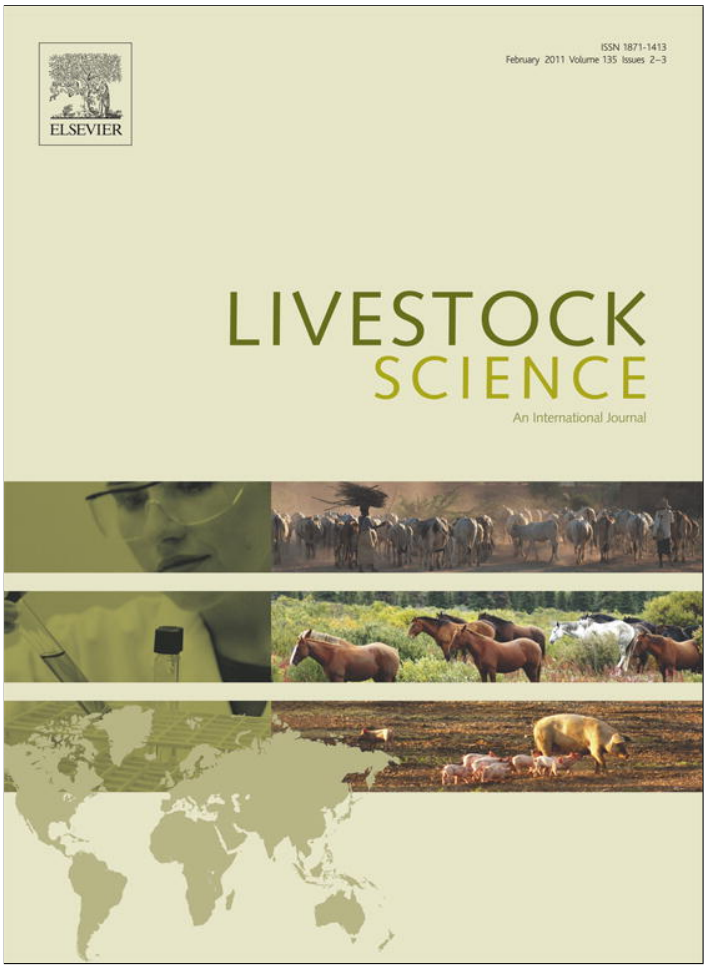

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# Genetic diversity and differentiation of Ankole cattle populations in Uganda inferred from microsatellite data

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ABSTRACT

A total of 304 individuals from eight Ankole cattle populations of Uganda were analysed based on 19 microsatellite markers to investigate genetic diversity, relationships and population structure. Across all loci, 200 alleles were observed. A high mean number of alleles (*MNA*) per locus, ranging from 5.89 to 6.79 per population, was observed. Polymorphic information content (PIC) ranged from 0.403 (*ILSTS013*) to 0.817 (*ILSTS036*), with an overall mean over all loci of 0.688. The average observed heterozygosity (*Ho*) was highest in Kaibanda (0.727) and lowest in Kituuha (0.648), while the expected heterozygosity (*He*) ranged from 0.722 (Nshaara) to 0.664 (Kituuha), though for all populations the differences were not significant. Significant deviations from Hardy–Weinberg proportions were observed over eight loci, however, all 152 loci–population combination tests were in equilibrium after Bonferroni correction. $F_{ST}$ estimates for all loci and between all populations were highly significant ($P<0.001$), suggesting little if any gene flow between the populations. *F*-statistics at respective loci among all populations were significant, with the exception of the $F_{IT}$. For all population pairs, $F_{ST}$ values were generally low, with an overall mean of $0.041 \pm 0.08$. Significant ($P<0.01$) inbreeding effect ($F_{IS}$) was detected in only the Nasasira population. The mean number of migrants per generation (*Nm*) across all populations was 3.82. Relationship analysis showed populations from the same geographical counties group together. These results illustrate at the molecular level the fairly wide genetic variation found within the Ankole breed and therefore the potential for genetic improvement.



## 1. Introduction

Ankole cattle are the main cattle breed in Uganda, but also exist in significant numbers in Rwanda, Burundi and North Western Tanzania (Wurzinger et al., 2007). The breed is used for milk and meat and is of great importance due to its tropical adaptation, local preferences, cultural and aesthetic value (Kugonza et al., 2005; Ndumu et al., 2008a). Indigenous cattle of Uganda are generally threatened by unplanned crossbreeding and upgrading to exotic breeds (Ssewannyana, 2004). Efforts need to be made to selectively conserve and improve upon their genetic potential for sustainable future performance. Breeding initiatives should include evaluation and selective improvement of the production traits of the Ankole breed and its crosses. One of the preliminary steps in selective breeding is the understanding of the genetic variation available within a breed.

Despite lack of consensus on procedures to assess genetic diversity using neutral markers (Meuwissen, 2009), there is

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still harmony on the need to continuously study this aspect. Allele diversity of microsatellite loci has enabled studies on genetic diversity, breed structure, relationships and introgression in cattle (Dadi et al., 2008), sheep (Muigai et al., 2009), goat (Chenyambuga et al., 2001; Li et al., 2002) and pig (Li et al., 2004). Analysis of genomic DNA variation within populations allows the optimum utilisation of genetic resources and allows efficient genetic improvement for production and conservation (Dadi et al., 2008). Previous work on microsatellite diversity and admixture in cattle (Freeman et al., 2005), identified areas of high diversity–population admixture in Western Asia at the crossroad of two putative centres of livestock domestication and population admixture. High level of genetic diversity in Eastern Africa and much less diversity in Western Africa and Europe have also been observed (Hanotte et al., 2002).

Indigenous breeds in developing countries are often inadequately documented and under utilised (Philipsson et al., 2006). This is the case in Uganda, where limited reliable information is available on the genetic diversity and performance of indigenous Ugandan cattle (DAGRIS, 2008; DAD-IS, 2008). The objective of this study was therefore, to assess genetic variability of Ugandan Ankole cattle populations.

## 2. Materials and methods

### 2.1. Study area

The study was conducted in Kazo and Nyabushozi counties of Mbarara district located about 250 km south west of Kampala, Uganda. This area has the highest concentration of the Ankole cattle in the world. Kijuma and Rwemikoma parishes in Kazo County; and Rushere, Rwamuranga and Nshaara parishes in Nyabushozi County were selected. The study sites are shown in Fig. 1.

### 2.2. Sampling procedure and DNA extraction

Blood samples were collected from 304 animals within 8 Ankole cattle populations in Mbarara district by jugular puncture using 10 ml disposable syringes and spread on FTA Whatman® filter paper (Whatman BioScience, Maidstone, UK). FTA purification reagent (Whatman® Bioscience) and Tris-EDTA (TE) buffer pH 7.6 were used to prepare DNA.

### 2.3. Microsatellite markers, PCR and genotyping

Nineteen cattle microsatellite markers recommended by FAO for measurement of domestic animal diversity were used: *AGLA293*, *BM1824*, *BM2113*, *ETH152*, *ETH225*, *ILSTS005*, *ILSTS006*, *ILSTS013*, *ILSTS023*, *ILSTS028*, *ILSTS033*, *ILSTS036*, *ILSTS50*, *ILSTS103*, *MGTG4B*, *TGLA53*, *TGLA122*, *TGLA126* and *TGLA227*. PCR amplification was performed in 10 μl reaction volumes containing 50 ng genomic DNA, 0.5 μl of 0.125 mM of each dNTP, 0.1 μl of each primer, 0.5 U *Taq* DNA polymerase and 1× PCR buffer containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 0.001% gelatine (Sigma), 0.25% Nonidet P40 (BDH, UK) and 2.0 mM $MgCl_2$. PCR amplifications were performed on a GeneAmp® PCR system 9700 (Applied Biosystems) thermocycler. Amplifications included an initial denaturing step of 5 min at 95 °C, followed by 35 cycles each of 30 s at 95 °C, 1 min at the primer annealing temperature (50–65 °C, Table S1) and 1 min extension at 72 °C. Final extension was for 10 min at 72 °C.

PCR products were resolved using an ABI-Prism 377 DNA Sequencer (Applied Biosystems). Microsatellite fragments were analysed using GeneScan™ 3.1.2 software (Applied Biosystems) and allele sizes were determined with the Genotyper™ 2.0 software (Applied Biosystems).

### 2.4. Statistical analyses

Allele frequencies and mean number of alleles (*MNA*) per locus were calculated using the Microsatellite Toolkit (Park, 2001). The *MNA* is the average number of alleles observed in a population over all loci genotyped and is a sensitive measure of genetic variability in comparison with the heterozygosity measure. Indeed, *MNA* is expected to be larger when the extent of polymorphism is higher, while the heterozygosity is hardly affected by the low-frequency alleles (Nei, 1987). An adjusted *MNA* estimate based on 20 individuals per population was obtained in this study using 250 replicates of resampling. This was to remove the effects of unequal subclass numbers and minimise the variation of *MNA* values between populations affected by sample size. Adjustment was done using Allele Sampler software (Mburu et al., 2003). Allelic polymorphic information content was calculated using CERVUS 1.0 software (Marshall et al., 1998).

Estimates of average observed heterozygosity ($H_O$) and expected unbiased heterozygosity ($H_E$) or gene diversity (Nei, 1987) for each population were obtained using GENEPOP version 3.3 software (Raymond and Rousset, 1995). The unbiased estimates of the exact *P*-values for Hardy–Weinberg equilibrium (HWE) tests at each locus and for each population were calculated using Markov chain randomisation (Guo and Thompson, 1992) using GENEPOP software. The parameters used were: dememorisation number, 1000; number of batches, 100; and number of iterations per batch, 1000. Results were adjusted to control type I error associated with multiple comparisons of data from a single sampling site using a sequential Bonferroni correction (Rice, 1989).

*F*-statistics ($F_{IT}$, $F_{IS}$, $F_{ST}$) (Weir and Cockerham, 1984), *R*-statistics (Rousset, 1996) for each locus, pairwise $F_{ST}$ between populations and the estimate of average inbreeding coefficient ($F_{IS}$) for each population were obtained and tested for significance according to the variance based method of Weir and Cockerham (1984) using the program FSTAT 2.9.3 software (Goudet, 2001). The contribution of individual loci significance per population to the overall significant deficit in heterozygotes was determined. Significance of population pairwise differentiation was also determined with Bonferroni corrections. The number of migrants per generation ($N_m = 1 - F_{ST}/4F_{ST}$) (Wright, 1969), an indirect estimate of gene flow, was calculated using the program GENETIX 4.03. Population isolation by distance was tested using Mantel tests (Mantel, 1967) by assessing for correlations between pairwise genetic and interpopulation land distances using the computer program ARLEQUIN 3.1. Statistical confidences of the tests were evaluated based on 10,000 random permutations.

$D_A$ genetic distance (Nei et al., 1983) between populations was calculated from allele frequencies considering nineteen markers. This measure was selected due to its superior

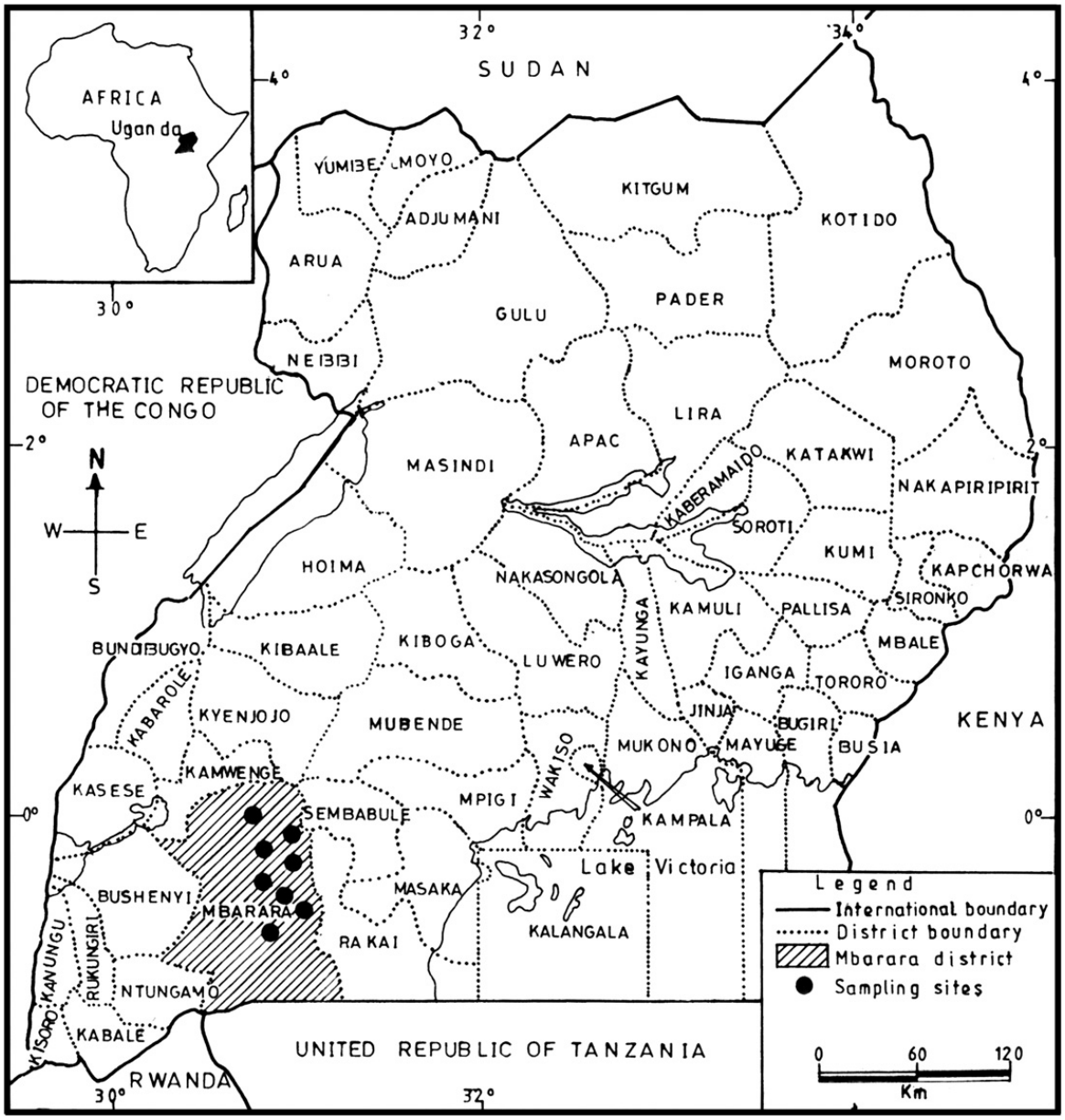


**Fig. 1.** Location of the cattle population sampling sites in Kazo and Nyabushozi, Uganda.

performance in phylogenetic tree construction when using microsatellites (Takezaki and Nei, 1996). The neighbour-joining (NJ) (Saitou and Nei, 1987) and the unweighted pair-group method with arithmetic mean (UPGMA) (Sneath and Sokal, 1973) algorithms were used to construct the dendrogram from the distance matrix, using the DISPAN program (Ota, 1993). The robustness of the dendrogram was evaluated by bootstrapping 1000 replicates of loci with replacement.

## 3. Results

### 3.1. Genetic diversity

A total of 200 different alleles were observed at the 19 loci in 304 samples from the eight Ankole cattle populations studied. The number of alleles per locus varied from six at *ETH152*, *BM1824*, *ILSTS013*, and *TGLA126* to 17 alleles at *ILSTS036*. Rwokusooka population had the highest mean number of alleles (*MNA*) per locus ($6.79 \pm 0.04$), while Kituuha had the lowest ($5.89 \pm 0.02$) (Table 1). Adjusted *MNA* for 20 individuals sub-sampled from each population was lower than *MNA* for that entire population, and ranged from $5.50 \pm 0.09$ (Kituuha) to $5.85 \pm 0.04$ (Nshaara). A total of 43 private alleles in 18 loci were observed across all populations. The highest proportion of these alleles (25.5%) was found in Kaibanda while Kasiisi and Nasasira had only one private allele each (Table 1).

The mean proportion of null alleles across all populations was 2.9%. The polymorphic information content (PIC) ranged from 0.403 (*ILSTS013*) to 0.817 (*ILSTS036*), with an overall mean over all loci of 0.688. The average observed heterozygosity (*Ho*) was highest in Kaibanda ($0.727 \pm 0.02$) and lowest in Kituuha ($0.648 \pm 0.02$), while the expected heterozygosity (*He*) ranged from $0.722 \pm 0.02$ (Nshaara) to $0.664 \pm$

**Table 1**
Within population genetic diversity expressed as mean number of alleles per locus (*MNA*) and inbreeding coefficients ($F_{IS}$) estimated from 19 microsatellite loci for each Ankole cattle population.

| County | Population | n | *MNA* ± s.e. for all animals | *MNA* ± s.e. for 20 animals [a] | Inbreeding coefficient [b] | Private alleles [c] |
|---|---|---|---|---|---|---|
| Kazo | Kasiisi | 28 | 6.05 ± 0.086 | 5.70 ± 0.081 | 0.020$^{ns}$ | 1 (1) |
| | Kituuha | 26 | 5.89 ± 0.020 | 5.50 ± 0.088 | 0.025$^{ns}$ | 7 (5) |
| | Nasasira | 44 | 6.37 ± 0.048 | 5.50 ± 0.039 | 0.054$^{**}$ | 1 (1) |
| | Rwokusooka | 52 | 6.79 ± 0.038 | 5.72 ± 0.031 | 0.010$^{ns}$ | 9 (7) |
| | Total | 150 | 6.28 ± 0.032 | 5.61 ± 0.010 | – | 18 |
| Nyabushozi | Kaibanda | 39 | 6.58 ± 0.064 | 5.74 ± 0.052 | −0.040$^{ns}$ | 11 (8) |
| | Mwesigye | 32 | 6.05 ± 0.057 | 5.55 ± 0.055 | −0.003$^{ns}$ | 4 (3) |
| | Nshaara | 41 | 6.74 ± 0.057 | 5.85 ± 0.043 | −0.001$^{ns}$ | 6 (6) |
| | Tayebwa | 42 | 6.37 ± 0.058 | 5.62 ± 0.047 | −0.025$^{ns}$ | 4 (3) |
| | Total | 154 | 6.44 ± 0.024 | 5.69 ± 0.011 | – | 25 |
| | Grand total | 304 | 6.36 ± 0.022 | 5.58 ± 0.019 | – | 43 (18) |

$^{ns}$Not significant ($P>0.05$).
[a] After 250 re-samplings.
[b] Significance level was calculated after 152,000 randomisations.
[c] Numbers in parentheses are loci on which the private alleles were located.
** Significant at $P<0.01$.

0.02 (Kituuha) (Table 2). For all populations, *Ho* and *He* were not significantly different ($\chi^2_{(8)} = 0.425$, $P = 1.00$). Significant ($P<0.01$) inbreeding effect ($F_{IS}$) was detected in only Nasasira population (Table 1).

### 3.2. Hardy–Weinberg equilibrium and linkage equilibrium

None of the studied populations deviated from Hardy–Weinberg equilibrium (HWE). However, there were loci significantly ($P<0.05$) deviating from HWE in different populations. This ranged from one locus (*TGLA227*) in Tayebwa (Table 2) to six in Kituuha (*ILSTS013*, *ILSTS023*, *ILSTS033*, *ILSTS050*, *TGLA53* and *TGLA122*). No single locus significantly deviated in all the eight populations. Seven of the 19 loci tested (*ILSTS023*, *ILSTS028*, *ILSTS033*, *TGLA122*, *ETH225*, *TGLA227* and *AGLA293*) significantly deviated from HWE ($P<0.05$). A total of 152 locus–population combinations (19 loci in eight populations) were tested for conformation to HWE. In total, 33 combinations deviated at the 5% level or lower, but after sequential Bonferroni correction, this number was reduced to 7, within the 5% statistical error range allowed.

### 3.3. Population differentiation and genetic relationships among populations

For each pair of populations, the differentiation index ($F_{ST}$) and their statistical significance are presented in Table 3. The Ankole cattle populations studied were all significantly differentiated ($P<0.001$). For all the population pairs, the mean $F_{ST}$ values were moderate, ranging from 0.018 for the Tayebwa–Nshaara pair to 0.073 between Kituuha and Mwesigye populations, and had an overall mean of 0.041 ± 0.08.

The results of *F*-statistics for respective loci across all populations are given in Table 4. The relative magnitude of gene differentiation among Ankole populations was 0.041, while the $F_{ST}$ estimator values for each locus were significantly ($P<0.01$) different from zero. $F_{IS}$ value was significantly ($P<0.001$) different from zero at *ILSTS023*, *ILSTS033*, *TGLA53*, *TGLA122* and *TGLA227*, while *TGLA126* and *ETH225* were significant at $P<0.05$. Significant ($P<0.01$) $F_{IT}$ was

**Table 2**
Within population genetic diversity expressed as average heterozygosity (*Ho* and *He*) and population and loci conformation to HWE from 19 microsatellite loci in Ankole cattle.

| County | Population | n | Observed heterozygosity | Expected heterozygosity | *P*-value [a] for population in HWE | Loci in HWE |
|---|---|---|---|---|---|---|
| | | | *Ho* ± s.e. | *He* ± s.e. | | |
| Kazo | Kasiisi | 28 | 0.696 ± 0.020 | 0.710 ± 0.030 | 0.001 | 14 |
| | Kituuha | 26 | 0.648 ± 0.023 | 0.664 ± 0.020 | 0.001 | 13 |
| | Nasasira | 44 | 0.672 ± 0.017 | 0.710 ± 0.026 | 0.001 | 14 |
| | Rwokusooka | 52 | 0.694 ± 0.015 | 0.700 ± 0.021 | 0.001 | 14 |
| | Total | 150 | 0.678 ± 0.002 | 0.696 ± 0.002 | 0.001 | – |
| Nyabushozi | Kaibanda | 39 | 0.727 ± 0.017 | 0.699 ± 0.027 | 0.001 | 15 |
| | Mwesigye | 32 | 0.712 ± 0.019 | 0.710 ± 0.026 | 0.05 | 16 |
| | Nshaara | 41 | 0.722 ± 0.017 | 0.722 ± 0.022 | 0.01 | 15 |
| | Tayebwa | 42 | 0.699 ± 0.017 | 0.683 ± 0.030 | 0.06 | 18 |
| | Total | 154 | 0.715 ± 0.001 | 0.704 ± 0.001 | 0.001 | – |
| | Grand total | 304 | 0.698 ± 0.006 | 0.727 ± 0.024 | 0.001 | 10 |

[a] Probability of population conformation to HWE.

**Table 3**
Pairwise genetic differentiation ($F_{ST}$) values (below diagonal) and their statistical significance (above diagonal) between the eight Ankole cattle populations estimated at 19 microsatellite loci.

| Population | KIT | KAS | NAS | RWO | KAI | TAY | MWE | NSH |
|---|---|---|---|---|---|---|---|---|
| Kituuha (KIT) | | *** | *** | *** | *** | *** | *** | *** |
| Kasiisi (KAS) | 0.06 | | ** | *** | *** | *** | *** | *** |
| Nasasira (NAS) | 0.07 | 0.03 | | *** | *** | *** | *** | *** |
| Rwokusooka (RWO) | 0.07 | 0.04 | 0.05 | | *** | *** | *** | *** |
| Kaibanda (KAI) | 0.06 | 0.04 | 0.04 | 0.041 | | *** | *** | *** |
| Tayebwa (TAY) | 0.07 | 0.04 | 0.04 | 0.039 | 0.03 | | *** | *** |
| Mwesigye (MWE) | 0.07 | 0.04 | 0.04 | 0.044 | 0.04 | 0.03 | | *** |
| Nshaara (NSH) | 0.06 | 0.03 | 0.03 | 0.029 | 0.03 | 0.02 | 0.021 | |

**$P<0.01$. ***$P<0.001$. Significance levels were obtained after 28,000 permutations. The indicative adjusted nominal level (5%) for multiple comparisons was 0.0018.

observed for *ILSTS023*, *ILSTS033*, *TGLA53* and *TGLA122*. These values corroborate the findings of loci deviations from HWE. Among the populations, only the Nasasira population had a significant $F_{IS}=0.054$ ($P<0.01$). $R_{ST}$ estimates across loci showed a similar trend as $F_{ST}$ (Table 4), supporting the geographical differentiation of the populations. A strong and highly significant correlation between genetic and geographical distances ($r=0.8734$, $P=0.0001$) was detected among the eight Ankole cattle populations following a Mantel's test with 10,000 randomisations. The mean number of migrants per generation ($Nm$) across all populations after correction for population size was 3.82.

Genetic relationships between Ankole populations determined using Nei's $D_A$ genetic distances (Nei et al., 1983) showed generally high distances between pairs of populations (Table 5). The smallest genetic distance (0.060) was found between Tayebwa and Nshaara populations while the highest distance (0.153) was observed between Kituuha and Mwesigye populations. The neighbour-joining method based on pairwise genetic distance matrix was used to construct a tree to represent the relationships between the populations (using $D_A$ genetic distance) (Fig. 2). The tree indicates that the cattle populations of Kazo County are different from those of Nyabushozi County. Two main clusters were identified from the tree.

The first cluster contained all the Kazo County populations: Kituuha, Kasiisi and Nasasira in the first group, while Rwokusooka and Nshaara population were in the second group. The second cluster had Nyabushozi County populations: Kaibanda alone in its group, while Tayebwa and Mwesigye were sub-clustered together. Nshaara population was clustered within Kazo County though it is geographically located in Nyabushozi County. The bootstrap values for the tree ranged from 45% to 68%. The standard genetic distance ($D_S$) matrix was also constructed (Table 6). The largest distance was observed between Kituuha and Tayebwa ($0.200\pm0.05$) while the lowest was between Mwesigye and Nshaara ($0.052\pm0.015$). An unrooted UPGMA tree showed a bootstrap value range of 29–66%, with two major clusters, of Kazo and Nyabushozi Counties, similar to the tree obtained using $D_A$ genetic distances. However, unlike the tree of $D_A$ distances, the UPGMA tree clustered Nshaara with Tayebwa with a bootstrap value of 60%.

## 4. Discussion

### 4.1. Genetic diversity

The understanding of the genetic variation available within a livestock breed was the focal point of this study. We sought to determine the status of Ugandan Ankole cattle at molecular marker level and found a broad range of genetic

**Table 4**
*F* and *R*-statistics and number of alleles for each of the 19 microsatellite loci using jack-knifing across the eight populations.

| Locus | $F_{IS}$ | $F_{IT}$ | $F_{ST}$ | $R_{ST}$ | Alleles |
|---|---|---|---|---|---|
| ILSTS005 | −0.016 (0.047)$^{ns}$ | 0.009 (0.050)$^{ns}$ | 0.024 (0.010)$^{**}$ | 0.038 | 9 |
| ILSTS006 | −0.078 (0.034)$^{ns}$ | −0.031 (0.037)$^{ns}$ | 0.044 (0.019)$^{**}$ | 0.011 | 10 |
| ILSTS013 | −0.039 (0.051)$^{ns}$ | −0.027 (0.058)$^{ns}$ | 0.011 (0.009)$^{**}$ | 0.014 | 6 |
| ILSTS023 | 0.149 (0.101)$^{***}$ | 0.206 (0.081)$^{**}$ | 0.068 (0.023)$^{**}$ | 0.048 | 7 |
| ILSTS028 | −0.044 (0.051)$^{ns}$ | −0.015 (0.046)$^{ns}$ | 0.028 (0.009)$^{**}$ | 0.019 | 16 |
| ILSTS033 | 0.187 (0.067)$^{***}$ | 0.231 (0.081)$^{**}$ | 0.052 (0.028)$^{**}$ | 0.089 | 7 |
| ILSTS36 | 0.023 (0.016)$^{ns}$ | 0.041 (0.017)$^{*}$ | 0.019 (0.005)$^{**}$ | 0.019 | 17 |
| ILSTS50 | −0.025 (0.040)$^{ns}$ | 0.012 (0.039)$^{ns}$ | 0.036 (0.012)$^{**}$ | 0.023 | 10 |
| TGLA53 | 0.085 (0.028)$^{***}$ | 0.128 (0.032)$^{**}$ | 0.047 (0.016)$^{**}$ | 0.033 | 19 |
| ILSTS103 | −0.052 (0.018)$^{ns}$ | 0.013 (0.024)$^{ns}$ | 0.062 (0.017)$^{**}$ | 0.076 | 8 |
| TGLA122 | 0.033 (0.040)$^{***}$ | 0.093 (0.055)$^{**}$ | 0.062 (0.017)$^{**}$ | 0.142 | 11 |
| TGLA126 | 0.023 (0.027)$^{*}$ | 0.065 (0.029)$^{*}$ | 0.043 (0.014)$^{**}$ | 0.095 | 6 |
| ETH152 | −0.085 (0.032)$^{ns}$ | −0.043 (0.047)$^{ns}$ | 0.039 (0.020)$^{**}$ | 0.018 | 6 |
| ETH225 | 0.032 (0.036)$^{*}$ | 0.070 (0.034)$^{*}$ | 0.040 (0.016)$^{**}$ | 0.063 | 11 |
| TGLA227 | 0.096 (0.048)$^{***}$ | 0.148 (0.046)$^{**}$ | 0.058 (0.008)$^{**}$ | 0.126 | 14 |
| AGLA293 | −0.094 (0.040)$^{ns}$ | −0.053 (0.038)$^{ns}$ | 0.037 (0.012)$^{**}$ | 0.054 | 13 |
| BM1824 | −0.022 (0.034)$^{ns}$ | 0.003 (0.029)$^{ns}$ | 0.025 (0.013)$^{**}$ | 0.053 | 6 |
| BM2113 | −0.054 (0.026)$^{ns}$ | −0.031 (0.024)$^{ns}$ | 0.021 (0.009)$^{**}$ | 0.008 | 13 |
| MGTG4B | −0.007 (0.049)$^{ns}$ | 0.042 (0.057)$^{ns}$ | 0.048 (0.017)$^{**}$ | 0.070 | 11 |

$^{ns}$Not significant. Significance levels were calculated after 19,000 randomisations. Standard errors are given in parentheses.
* $P<0.05$.
** $P<0.01$.
*** $P<0.001$.

**Table 5**
Nei's $D_A$ genetic distance matrix among eight Ankole cattle populations estimated from 19 microsatellite loci.

| Population | KIT | KAS | NAS | RWO | KAI | TAY | MWE |
|---|---|---|---|---|---|---|---|
| Kituuha (KIT) | | | | | | | |
| Kasiisi (KAS) | 0.139 | | | | | | |
| Nasasira (NAS) | 0.132 | 0.090 | | | | | |
| Rwokusooka (RWO) | 0.141 | 0.110 | 0.094 | | | | |
| Kaibanda (KAI) | 0.130 | 0.124 | 0.091 | 0.089 | | | |
| Tayebwa (TAY) | 0.131 | 0.103 | 0.075 | 0.087 | 0.069 | | |
| Mwesigye (MWE) | 0.153 | 0.113 | 0.094 | 0.106 | 0.098 | 0.076 | |
| Nshaara (NSH) | 0.133 | 0.098 | 0.080 | 0.081 | 0.078 | 0.060 | 0.067 |

diversity at the nineteen loci, which indicates a rich gene pool within this breed. Two hundred alleles observed across all loci in 304 animals compare well with 207 alleles in 472 individuals from 11 overlapping Ankole populations (Ndumu et al., 2008b). Within population genetic variability (*He*) of 0.664–0.722 was higher than 0.432–0.658 (MacHugh et al., 1997) and 0.45–0.69 (Kantanen et al., 2000) reported among African and European cattle breeds respectively. However, *He* values of this study do not differ from those in other Ankole populations from an earlier study, except Kagera in Tanzania (Ndumu et al., 2008b). The overall *He* ($0.727 \pm 0.024$) for the Ankole breed was much higher than $0.662 \pm 0.030$ which was observed within the Spanish Alberes cattle breed (Casellas et al., 2004). As would be expected, Nshaara population which had the highest number of migrants (5.4) compared to the other populations, also had most genetic variability.

The average number of alleles follows a pattern similar to that described for *He*. The mean number of alleles per locus (*MNA*) was $6.36 \pm 0.022$ and average observed heterozygosity, $H_O$, was 69.8%. These levels of within population genetic variability are close to the values obtained in the Mbarara population (*MNA* 6.8, $H_O = 73.7\%$, Ndumu et al., 2008b) West African cattle (*MNA* 6.7, $H_O = 64.3\%$, MacHugh et al., 1997) but were lower than Bhutan cattle breeds (*MNA* 7.2, $H_O = 67\%$, Dorji et al., 2003). Similar results have been described for various European cattle breeds (Schmid et al., 1999). Results show that the mean frequency of null alleles observed was lower than in other cattle studies. Ankole cattle therefore show a relatively high level of diversity.

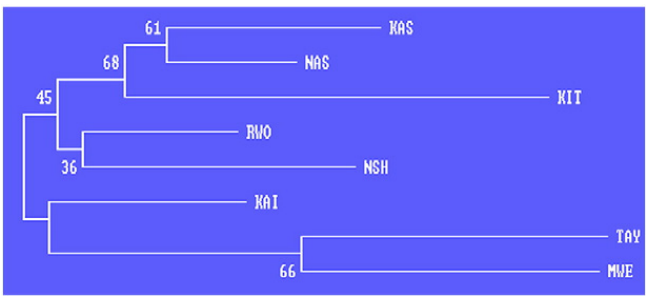


**Fig. 2.** Unrooted neighbour-joining tree showing the genetic relationships among the Ankole cattle populations using $D_A$ genetic distance calculated from 19 microsatellite loci. The numbers at the node indicate bootstrap values (1000 re-sampling). The abbreviated population names are: Kasiisi, Nasasira, Kituuha, Rwokusooka, Nshaara, Kaibanda, Tayebwa and Mwesigye.

### 4.2. Genetic differentiation among Ankole cattle populations

The non-significant $F_{IS}$ and $F_{IT}$ estimates in most populations indicate that generally, there was no inbreeding in the studied populations. However, inbreeding effect was detected in Nasasira population, where five loci were in HW disequilibrium. After interviewing the owner, we can likely attribute inbreeding to the selection of replacement bulls from within the populations in attempt to select for dark red *bihogo* colour (Infield et al., 2003). The non-significance of the *F*-parameters could also be a result of gene flow with other populations.

The genetic differentiation analysis ($F_{ST}$) showed that the Ankole cattle populations were genetically distinct, with an average of 4.6% of variation being between populations. This value for within breed variation is similar to other findings for this breed (Ndumu et al., 2008b), and quite high when compared to the 6% between 12 African cattle breeds of Cameroon and Nigeria (Ibeagha-Awemu and Erhardt, 2006). The level of differentiation was also much higher than 2.6% found between three populations of the Spanish Alberes cattle breed (Casellas et al., 2004) and 3.5% found between Belgian cattle populations (Mommens et al., 1999). This could be explained by differences in population history and management.

Genetic differentiation studies of European cattle breeds reported $F_{ST}$ values of 0.068 in Southern Europe (Jordana et al., 2003), 0.090 among Swiss cattle (Schmid et al., 1999), 0.107 in 20 Northern Europe breeds (Kantanen et al., 2000) and 0.112 found in seven breeds from around the continent (MacHugh et al., 1998). Levels of differentiation observed in Ankole cattle populations of this study that ranged between 0.018 and 0.073 are comparable to the one found among European cattle breeds or among breeds of other livestock species (e.g. $F_{ST} = 0.105$ in Asian goats (Barker et al., 2001) and 0.143 in Chinese goats (Li et al., 2002)). These results hence show that the populations studied are very distinct from each other and not just sub-populations as reported by others (e.g. Ndumu et al., 2008b), the cause of which is not apparent.

Significant ($P<0.001$) genetic differentiation ($F_{ST}$) was identified between all population pairs although the index values were moderate (0.018–0.073) and in most cases, corresponded to an increase of geographical distance between farms, with exception of Kituuha and Nshaara populations which had the lowest $F_{ST}$ value though farthest, geographically apart. This anomaly is attributed to the Nshaara populations being recently assembled with animals acquired from surrounding areas. Genetic heterogeneity among livestock populations found over relatively short geographical distances have also been observed in European cattle (Jordana and Piedrafita, 1996; Casellas et al., 2004). For this study, genetic differentiation between populations is further illustrated by the high and significant correlation between geographical and genetic distances showed by the Mantel test.

The estimated number of migrants per generation ($N_m$) between populations was generally low (3.2), indicating that there is minimal exchange of genetic material, between populations, though this level of migration is expected to maintain the genetic differentiation observed between the populations. It is also likely that bulls are acquired from

**Table 6**
Standard genetic distance matrix (below diagonal) among eight Ankole cattle populations estimated from 19 microsatellite loci.

| Population [a] | KIT | KAS | NAS | RWO | KAI | TAY | MWE | NSH |
|---|---|---|---|---|---|---|---|---|
| Kituuha (KIT) | 1 | 0.031 | 0.042 | 0.041 | 0.039 | 0.050 | 0.044 | 0.036 |
| Kasiisi (KAS) | 0.128 | 1 | 0.020 | 0.017 | 0.026 | 0.035 | 0.027 | 0.019 |
| Nasasira (NAS) | 0.187 | 0.086 | 1 | 0.028 | 0.018 | 0.025 | 0.028 | 0.020 |
| Rwokusooka (RWO) | 0.168 | 0.098 | 0.129 | 1 | 0.024 | 0.031 | 0.028 | 0.020 |
| Kaibanda (KAI) | 0.145 | 0.107 | 0.093 | 0.102 | 1 | 0.026 | 0.025 | 0.020 |
| Tayebwa (TAY) | 0.200 | 0.118 | 0.105 | 0.111 | 0.066 | 1 | 0.043 | 0.026 |
| Mwesigye (MWE) | 0.169 | 0.107 | 0.126 | 0.110 | 0.116 | 0.114 | 1 | 0.015 |
| Nshaara (NSH) | 0.131 | 0.069 | 0.085 | 0.070 | 0.071 | 0.063 | 0.052 | 1 |

[a] Values above the diagonal are standard errors.

specific populations. The number of migrants per generation among Ankole cattle is lower than those reported by others for this breed (Ndumu et al., 2008b), but compares well with findings in other cattle breeds such as the Pyrenean (Jordana and Piedrafita, 1996) and Alberes cattle (Casellas et al., 2004), which exist on a geographical area, smaller than that used in our study.

The neighbour-joining tree constructed using a matrix of $D_A$ genetic distances among the eight Ankole cattle populations showed that the populations within each county were closer to each other than to the populations of another county. It is plausible that the population from Nshaara ranch (owned by government), and which was constituted recently, contains animals originating from the two counties and that most of the animals sampled for the current study had mainly originated from Kazo County.

Ndumu et al. (2008b) found that Ankole cattle in north Mbarara and Luweero districts of Uganda clustered closest. Ndumu et al. (2008b) also observed very low divergence between populations from different countries, yet in this study, populations from within Mbarara showed high divergence (Fig. 2). These findings imply that Ankole cattle populations outside Uganda (in Rwanda and Tanzania especially) are not very distinct and uncontrolled mating between them may be common. Many Ankole populations outside Uganda are still managed under pastoralism (Wurzinger et al., 2007), and according to Ndumu et al. (2008b), the number of migrants exchanged between these populations is high.

## 5. Conclusion

Ankole cattle of Mbarara show a high within population diversity negligible in breeding and significant between population genetic differentiation, comparatively higher than other Ankole cattle populations outside Uganda. This contrasts with Ndumu et al. (2008b) who observed homogeneity in Ugandan Ankole cattle populations. The populations in Uganda are an important genetic resource that should be conserved for breed improvement programs that have been proposed for this breed. Conservation efforts should focus on the populations in different counties where the Ankole cattle are being kept, and not assume that the populations are homogeneous as earlier presumed. Further research should determine the level of introgression of other indigenous Ugandan breeds into the Ankole populations, so as to further inform the Ankole cattle conservation strategy.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.livsci.2010.06.158.

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