

ASSESSMENT OF THE GENETIC DIVERSITY AND RELATIONSHIPS AMONG AFRICAN FAT-TAILED SHEEP: PRELIMINARY RESULTS

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SUMMARY

Fat-tailed sheep are amongst the most widely distributed sheep on the African continent being the main sheep populations in a large part of Northern Africa (from Egypt to Algeria) and in the Eastern and Southern African regions (from Eritrea to South Africa). Genetic relationship between fat-tailed sheep populations is largely unknown. Twelve indigenous African fat-tailed and fat-rump sheep breeds - Menz, Tekur, Afar, Wello, Horro (all from Ethiopia), Blackhead Persian (Uganda), Red Maasai (Kenya), Pedi and Blackhead Persian (South Africa), Tswana (Botswana), Damara (Namibia) and Ossimi (Egypt) were studied with 23 autosomal microsatellite loci. Non-African reference breeds included three fat-tailed sheep populations (Karakul from Central Asia but sampled in Namibia, Lanzhou from Central China and Awassi from Syria), three thin-tailed breeds from West Africa (West African Dwarf and Uda from Nigeria, Maure from Mali) and three thin-tailed breeds from Europe (North Ronaldsay, Dorset and Swaledale). Genetic diversity was high within all breeds with observed heterozygosity ranging from 0.656 ± 0.044 (Pedi) to 0.776 ± 0.024 (Ossimi). The standard genetic distance D_s ranged from 0.083 ± 0.87 between North Ronaldsay and Wello to 0.648 ± 0.139 between Lanzhou and Pedi. Average genetic distances between fat-tailed sheep populations of Eastern and Southern Africa was 0.342 ± 0.0345 . Phylogenetic tree (neighbour-joining, D_s) and multi-dimensional scaling analysis showed a clear separation of the Eastern and Southern African fat-tailed populations from all the other sheep populations. On the other hand, there is no clear distinction between the fat-tailed sheep populations from outside sub-Saharan Africa and the thin-tailed sheep populations from Africa and Europe.

Keywords: African indigenous sheep, microsatellites, genetic diversity

INTRODUCTION

It is estimated that the African continent is hosting 209 million head of sheep (FAO, 1996). They are a particularly important resource for African farmers providing meat, milk, manure, fibers and hides. However, introduction of exotic sheep breeds, disease, droughts and civil strife are threatening these potentially unique genetic resources with extinction. African sheep are thought to have entered the continent following two routes, *via* the Isthmus of Suez and the Horn of Africa. Two main types of sheep, the fat- and thin-tailed sheep, are currently found on the continent (Mason, 1969).

Fat-tailed sheep populations are found on the Northern part of the continent, from Egypt to Algeria and on the Eastern and Southern regions of the continent, from Eritrea to South Africa, making them the most widely distributed sheep in Africa. The thin-tailed sheep are found mainly in Morocco, Sudan and in Western Africa from Senegal to Nigeria.

Awareness of the value of genetic resources has encouraged studies of the genetic diversity present in different animal species. Recent studies have successfully used microsatellites to characterize the genetic variation among livestock including sheep (e.g. Hanotte *et al.*, 2000, Hanslik *et al.* 2000, Arranz *et al.*, 1998, MacHugh *et al.*, 1997; Parsons *et al.*, 1996, Buchanan *et al.*, 1994). The objective of this study was to determine the genetic diversity of sub-Saharan African sheep populations in order to facilitate their rational development, utilization and conservation. A total of 23 microsatellite genetic markers were used to study 21 sheep populations that included 12 African fat-tailed populations.

MATERIALS AND METHODS

Populations and DNA extraction

Twelve fat-tailed and fat-rumped breeds indigenous to Africa were studied. Nine other breeds were included in the study to serve as reference breeds. Details of breeds, country of origin and phenotype are shown in Table 1. Blood or hair samples were collected from 35 – 40 individuals. Efforts to ensure that individuals sampled were not closely related involved selecting only a small number of animals from each herd and questioning the farmers on the genealogy of each animal. DNA was extracted from the blood or hair samples and was amplified with the microsatellite loci listed in Table 2. PCR products were analyzed by denaturing polyacrylamide gel electrophoresis using an automated DNA sequencer (ABI 377). The results were analysed using GENESCAN™ 6.7.2 (Ver 3.0) and the GENOTYPER™ (Ver 2.0) softwares (Applied Biosystems).

Statistical analysis

Allele frequencies were calculated using the programme Excel Micro 97 (kindly provided by Stephen Park, spark@tdc.ie). Observed heterozygosities (H_o), standard genetic distances (D_s) and gene diversities between breeds were calculated using the DISPAN program (Ota, 1993). Neighbour-joining trees were constructed and the reliability of the tree obtained was examined by a bootstrap test with 1000 replicates. Multi-dimensional scaling was done using the program XLSTATS (ver 4.3, <http://www.xlstat.com>).

Table 1: Observed heterozygosity (H_o) and mean number of alleles per locus

Populations ¹	Type	Country	$H_o \pm S.E.$	Mean number of alleles per locus
African Fat-tailed	Fat-tailed hair	Namibia	0.684 ± 0.038	7.5 ± 0.75
Damara	Fat-tailed hair	Botswana	0.689 ± 0.053	6.9 ± 0.64
Tswana	Fat-tailed hair	South Africa	0.656 ± 0.044	6.3 ± 0.67
Pedi	Fat-tailed hair	Kenya	0.730 ± 0.033	7.6 ± 0.46
Red Maasai	Fat-tailed hair	Ethiopia	0.682 ± 0.057	7.1 ± 0.72
Afar	Fat-tailed hair	Ethiopia	0.678 ± 0.035	6.5 ± 0.48
Horro	Fat-tailed hair	Ethiopia	0.720 ± 0.030	6.9 ± 0.62
Menz	Fat-tailed coarse wool	Ethiopia	0.718 ± 0.043	7.4 ± 0.61
Tekur	Fat-tailed coarse wool	Ethiopia	0.731 ± 0.036	7.1 ± 0.54
Wello	Fat-tailed	Ethiopia	0.776 ± 0.024	7.1 ± 0.54
Ossimi	Fat-tail wool	Egypt	0.663 ± 0.038	5.9 ± 0.61
Blackhead Persian-SA	Fat-rump hair	South Africa	0.742 ± 0.025	8.0 ± 0.5
Blackhead Persian-UG	Fat-rump hair	Uganda	0.706 ± 0.236	7.0 ± 0.17
Reference breeds		Mean	0.706 ± 0.236	7.0 ± 0.17
Karakul	Fat-tailed fur	Central Asia ²	0.685 ± 0.035	7.1 ± 0.68
Awassi	Fat-tailed fleecce wool	Syria	0.717 ± 0.037	7.1 ± 0.65
Lanzhou	Fat-tailed	China	0.734 ± 0.039	8.7 ± 0.76
Swaledale	Short-tailed wool	United Kingdom	0.701 ± 0.043	7.1 ± 0.67
Dorset	Thin-tailed	United Kingdom	0.717 ± 0.040	7.1 ± 0.66
North Ronaldsay	Thin-tailed	United Kingdom	0.705 ± 0.029	6.9 ± 0.56
West African Dwarf (Djallonké)	Thin-tailed hair	Nigeria	0.723 ± 0.025	7.5 ± 0.67
Uda	Thin-tailed hair	Nigeria	0.748 ± 0.029	7.6 ± 0.66
Maure	Thin-tailed hair	Mali	0.724 ± 0.032	7.6 ± 0.8
		Mean	0.717 ± 0.240	7.4 ± 0.18

¹Adapted from Epstein (1971) and Rege *et al.* (1996).

²Sampled in Namibia

RESULTS

Genetic diversity and genetic distances:

A total of 286 different alleles were scored. The mean number of alleles and the average heterozygosities in each sheep population are indicated at Table 1. The population with the least allelic diversity was the Blackhead Persian from South Africa, with a mean number of alleles of 5.9 ± 0.61 , while that with the highest was Lanzhou from China with an average 8.7 ± 0.76 alleles *per locus*. Amongst the fat-tailed sheep of Eastern and Southern Africa the Blackhead Persian from Uganda had

Table 2. Autosomal microsatellite loci

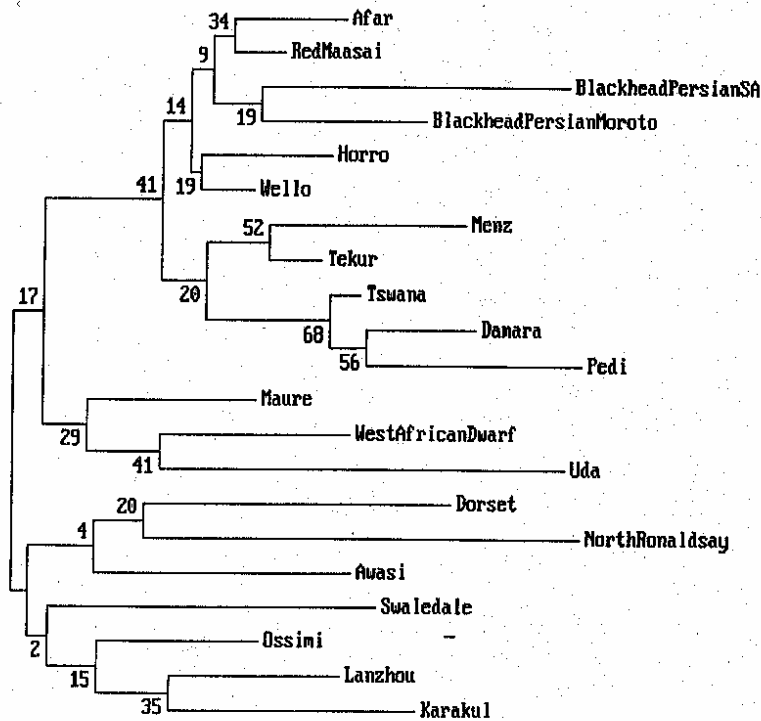
Locus	Chromosome	Allele size (bp)	Forward 5'-3' primer	Reverse 3'-5' primer
MCM42	9	84 - 112	cat ctt tca aaa gaa ctc aga aag tg	ctt gga atc ctt cct aac ttt cgg
MAF65	15	111 - 139	aaa ggc cag agt atg caa tta gga g	cca ctc ctc tga gaa tat aac atg
OARFCB11	2	121-149	gca agc agg ttc ttt acc act agc acc	gga ctc aac tca caa gtt gat ata tct atc ac
MCM527	5	155 - 191	gtc cat tgc ctc aaa tca att c	aaa cca ctt gac tac tcc cca a
MAF214	16	186 - 204	aat gca gga gat ctg agg cag gga cg	ggg tga tct tag gga ggt ttt gga gg
HSC	unknown	267 - 301	ctg cca atg cag aga cac aag a	gtc tgt ctc ctg tct tgt cat c
OARFCB20	2	88 - 158	gga aaa ccc cca tat ata cct ata c	aaa tgt gtt taa gat tcc ata cat gtt
ILSTS049	3	150 - 174	caa ttt tct tgt ctc tcc cc	gct gaa tct tgt caa aca gg
OARAE129	5	134 - 164	aat cca gtg tgt gaa aga cta atc cag	gta gat caa gat ata gaa tat ttt tca aca cc
OARFCB129	3	105 - 125	ggc act tag cag cag cag cat cc	cat caa gag atg aat gag taa aga aga tg
ILSTS056	10	145 - 175	gct act gag tga tgg tag gg	aat ata gcc ctg gag gat gg
ILSTS005	7	186 - 218	gg aag caa tga aat cta tag cc	tgt tct gvg agt tgg taa gc
ILSTS019	21	144 - 202	aag gaa cct cat gta gaa gc	act ttt gga ccc tgt agt gc
SR-CRSP-5	18	139 - 153	gga ctc tac caa ctg agc tac aag	tga aat gaa gct aaa gca atg c
TGLA53	12	117 - 165	gct ttc aga aat atg ttt cat tca	atc ttc aca tga tat tac agc aga
OARJMP29	24	120 - 150	gta tac acg tgg aca cag ctt tgt ac	gaa gfg gca aga ttc aga ggg gaa g
ILSTS017	x	82 - 126	gtc cct aaa atc gaa atg cc	gca tct cta taa cct gtt cc
BM1818	20	220 - 250	agc tgg gaa tat aac caa agg	agt gct ttc aag gtc cat gc
MAF209	17	98 - 136	gat cac aaa aag ttg gat aca acc gfg g	tca tgc act taa gta tgt agg atg ctg
OARFCB304	19	98 - 164	ccc tag gag ctt tca ata aag aat cgg	cgc tgc tgt caa ctg ggt cag gg
MAF035	23	90 - 118	tca aga att ttg gag cac aat tct gg	agt tac aaa tgc aag cat cat acc tg
ILSTS044	1	98 - 132	agt cac cca aaa gta act gg	aca tgt tgt att cca agt gc
BM827	3	92 - 104	ggg ctg gtc gta tgc tga g	ggt gga ctt gct gaa gfg acc

the highest allelic diversity of 8.0 ± 0.5 (Table 1). On average the allelic diversity was lower amongst the African fat-tailed sheep compared to the breeds of reference (Table 1).

The observed heterozygosities were relatively high for all breeds and ranged from 0.656 ± 0.044 for Pedi to 0.776 ± 0.024 for Ossimi. Average genetic distance between fat-tailed sheep population of Eastern and Southern Africa was 0.342 ± 0.035 . The pooled heterozygosities for the African fat-tailed sheep was 0.706 ± 0.236 . It was slightly lower than the pooled heterozygosities of the reference breeds which was 0.717 ± 0.240 .

The standard genetic distance (D_s) ranged from 0.083 ± 0.870 between North Ronaldsay and Wello to 0.648 ± 0.139 between Lanzhou and Pedi (Table 3). A neighbour-joining tree constructed from genetic distances (D_s) showed two main clusters of sheep (Figure 1). The first cluster included the North African fat-tailed sheep Ossimi and the Asian, Middle Eastern and European breeds of reference. All other African breeds clustered together in the second cluster with the Eastern and Southern fat-tailed breeds being more closely related to each other than to the West African thin-tailed breeds. With the exception of the two Blackhead Persian populations studied, the Eastern and Southern African fat-tailed populations are clearly separated. Similar results are obtained with the multidimensional scaling analysis where the Eastern and Southern African fat-tailed sheep group together (Figure 2).

Figure 1: Genetic relationships among the sheep breeds (neighbour-joining tree, D_s , 1000 re-sampling).



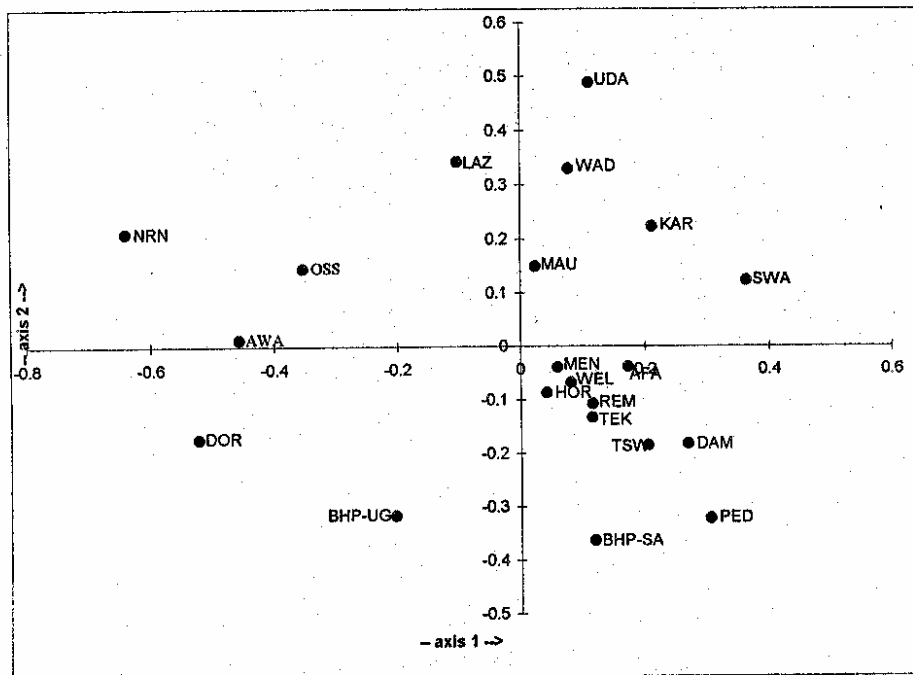


Figure 2: Multidimensional scaling analysis of 21 sheep populations with 23 microsatellite loci

AFA- Afar, Dam- Damara, HOR- Horro, MEN- Menz, PED- Pedi, SWA- Swaledale, TEK- Tekur, TSW- Tswana, WEL- Wello, REM- Red Maasai, AWA- Awasi, LAZ- Lanzhou, DOR- Dorset, BHP-SA- Blackhead Persian- South Africa, WAD- West African Dwarf, BHP-UG-Blackhead Persian-Uganda, UDA- Uda, KAR- Karakul, OSS- Ossimi, MAU- Maure, NRN- North Ronaldsay

DISCUSSION

The mean number of alleles per locus and the observed heterozygosity for all the breeds studied were relatively high suggesting high levels of genetic diversity within the breeds. The population with the highest allelic diversity was the Blackhead Persian of Uganda which also showed a relatively high observed heterozygosity (Table 1). This population, found in North-Western Uganda, is today fairly isolated but its past history is unknown. The population with the lowest diversity and the second lowest H_o value amongst the fat-tailed sheep of Africa was the Blackhead Persian sampled in South Africa. This is relatively surprising because this breed has been used in the development of other breeds such as the Dorper, White Wool Mountain and Bezuidehout Africander (Rege *et al.*, 1996). Its low diversity could be a consequence of the small size of its founding population in South Africa (Epstein, 1971).

The phylogenetic tree (Figure 1) and multidimensional scaling (Figure 2) showed a clear separation of the Eastern and Southern African fat-tailed sheep from all the other populations of sheep studied. It should however be noted that the bootstrap values of this tree are generally very low and caution should be taken in the interpretation of these results. More markers and breeds could further clarify these results. Nevertheless they indicate that most of the present day sheep breeds are the result of different genetic influences (e.g European, Near Eastern and Asian fat-tailed sheep breeds) with the possible exception of the East and South African fat-tailed sheep.

These preliminary results support two separate origins for the fat-sheep populations of Africa, one through the Isthmus of Suez and another through the Horn of Africa. However, it cannot be fully excluded that the ancestral populations of the Ossimi could have entered Africa through the Horn and subsequently migrated to the North where they would have crossbred with the local thin-tailed sheep.

Table 3. Standard genetic distances (Ds) between the 12 African fat-tailed sheep and 9 breeds of reference

	AFA	DAM	HOR	MEN	PED	SWA	TEK	TSW	WEL	REM	AWA	LAZ	DOR	BHP- SA	WAD	BHP- UG	KAR	OSS	MAU	NRN	
AFA	0.078	0.040	0.129	0.063	0.125	0.057	0.061	0.031	0.028	0.126	0.084	0.148	0.148	0.057	0.062	0.052	0.152	0.092	0.084	0.070	0.121
DAM	0.264	0.096	0.073	0.044	0.109	0.054	0.032	0.071	0.070	0.111	0.101	0.117	0.117	0.076	0.116	0.076	0.138	0.132	0.099	0.063	0.129
HOR	0.144	0.339	-	0.101	0.096	0.101	0.064	0.071	0.035	0.045	0.077	0.093	0.091	0.071	0.082	0.065	0.141	0.111	0.085	0.081	0.125
MEN	0.285	0.254	0.237	-	0.074	0.101	0.079	0.087	0.091	0.101	0.077	0.111	0.108	0.094	0.109	0.130	0.127	0.102	0.099	0.114	0.127
PED	0.294	0.176	0.389	0.366	-	0.119	0.050	0.034	0.078	0.067	0.150	0.139	0.120	0.092	0.102	0.085	0.180	0.152	0.104	0.099	0.168
SWA	0.363	0.407	0.361	0.340	0.518	-	0.110	0.087	0.098	0.096	0.146	0.069	0.109	0.134	0.107	0.115	0.134	0.120	0.075	0.101	0.103
TEK	0.189	0.227	0.187	0.135	0.277	0.351	-	0.034	0.053	0.051	0.124	0.135	0.092	0.063	0.083	0.066	0.141	0.160	0.077	0.071	0.134
TSW	0.195	0.102	0.215	0.231	0.146	0.309	0.130	-	0.061	0.056	0.102	0.089	0.105	0.057	0.094	0.061	0.144	0.110	0.109	0.062	0.101
WEL	0.110	0.253	0.100	0.173	0.314	0.325	0.115	0.183	-	0.034	0.090	0.077	0.073	0.051	0.080	0.059	0.133	0.091	0.091	0.073	0.084
REM	0.088	0.244	0.137	0.251	0.249	0.328	0.132	0.156	0.092	-	0.102	0.088	0.111	0.057	0.077	0.061	0.127	0.094	0.094	0.084	0.103
AWA	0.438	0.477	0.286	0.267	0.597	0.438	0.384	0.424	0.306	0.360	-	0.099	0.106	0.141	0.132	0.111	0.151	0.126	0.126	0.116	0.137
LAZ	0.396	0.496	0.343	0.391	0.648	0.313	0.423	0.440	0.339	0.337	0.243	-	0.078	0.148	0.098	0.107	0.114	0.063	0.063	0.083	0.099
DOR	0.539	0.405	0.350	0.335	0.489	0.390	0.408	0.440	0.341	0.436	0.313	0.258	-	0.109	0.119	0.128	0.131	0.140	0.140	0.123	0.120
BHP-SA	0.301	0.323	0.298	0.398	0.285	0.565	0.288	0.287	0.224	0.215	0.521	0.544	0.511	-	0.109	0.068	0.156	0.163	0.163	0.131	0.157
WAD	0.296	0.489	0.263	0.388	0.485	0.405	0.320	0.391	0.278	0.266	0.414	0.366	0.498	0.440	-	0.084	0.098	0.114	0.114	0.090	0.145
BHP-UG	0.180	0.356	0.203	0.359	0.316	0.501	0.226	0.253	0.173	0.142	0.427	0.446	0.457	0.257	0.285	-	0.154	0.101	0.101	0.095	0.146
UDA	0.492	0.526	0.526	0.468	0.526	0.492	0.492	0.561	0.406	0.443	0.435	0.426	0.442	0.551	0.322	0.465	-	0.124	0.124	0.141	0.141
KAR	0.311	0.523	0.411	0.400	0.560	0.402	0.505	0.431	0.338	0.308	0.364	0.226	0.547	0.489	0.376	0.391	0.516	-	0.124	0.124	0.148
OSS	0.333	0.404	0.404	0.352	0.348	0.386	0.294	0.311	0.260	0.267	0.301	0.305	0.247	0.466	0.440	0.277	0.343	0.234	-	0.059	0.113
MAU	0.249	0.304	0.238	0.332	0.391	0.384	0.273	0.280	0.249	0.264	0.273	0.333	0.456	0.462	0.278	0.298	0.306	0.400	0.214	-	0.128
NRN	0.121	0.129	0.125	0.127	0.168	0.103	0.134	0.104	0.083	0.103	0.137	0.099	0.120	0.156	0.569	0.603	0.495	0.467	0.404	0.432	-

Genetic distances (Ds) below the diagonal, standard errors above the diagonal

AFA - Afar, DAM - Damara, HOR - Horro, MEN - Menz, PED - Pedi, SWA - Swaledale, TEK - Tekur, TSW - Tswana, WEL - Welio, REM - Red Maasai, AWA - Awasi, LAZ - Lanzhou, DOR - Dorset, BHP-SA - Blackhead Persian - South Africa, WAD - West African Dwarf, BHP-UG - Blackhead Persian - Uganda, UDA - Uda, KAR - Karakul, OSS - Ossimi, MAU - Maure, NRN - North Ronaldsay

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REFERENCES

- Arranz J.J., Bayon Y. and Primitivo F.S. (1998). Genetic relationships among Spanish breeds using microsatellites. *Animal Genetics* 29, 435-440.
- Buchanan F.C., Adams L.J., Littlejohn J.F., Maddox J.F. and Crawford A.M. (1994). Determination of evolutionary relationships among sheep breeds using microsatellites. *Genomics* 22, 397-403.
- Epstein H. (1971). *The origin of the domestic animals of Africa*, Africana Publishing Corporation, New York.
- FAO (1996). *FAO Production Yearbook-1996*, FAO, Rome.
- Hanotte O., Tawah C.L., Bradley D.G., Okomo M., Verjee Y., Ochieng J. and Rege J.E.O. (2000). Geographic distribution of a taurine *Bos taurus* and an indicine *Bos indicus* Y specific allele amongst sub-Saharan African cattle breeds. *Molecular Ecology* 9, 387-396.
- Hanslik S., Harr B., Brem G. and Schlotterer C. (2000). Microsatellite Data analysis reveals substantial genetic differentiation between contemporary New World and Old World Holstein Friesian populations. *Animal Genetics* 31, 31-38.
- MacHugh D.E., Shriver M.D., Loftus R.T., Cunningham P. and Bradley D.G. (1997). Microsatellite DNA variation and the evolution, domestication and phylogeography of taurine and zebu cattle (*Bos taurus* and *Bos indicus*). *Genetics* 146, 1071-1086
- Mason I.L. (1969). *A World Dictionary of Livestock Breeds, Types and Varieties*, 2 edition. Commonwealth Agricultural Bureaux: Farnham Royal, Bucks, England
- Ota T. (1993). *Dispan: Genetic Distance and Phylogenetic Analysis*, Pennsylvania State University, PA.
- Parsons Y.M., Cooper D.W. and Piper L.R. (1996). Genetic variation in Austrian Merino sheep. *Animal Genetics* 27, 223-228.
- Rege J.E.O., Yapi-Gnaore C.V. and Tawah C.L. (1996). The indigenous domestic ruminant genetic resources of Africa. *Invited paper presented at the 2nd All Africa Conference on Animal Agriculture, Pretoria, South Africa (1st-4th April 1996)*.