GENETIC RELATIONSHIPS BETWEEN NATIVE SHEEP BREEDS IN NIGERIA BASED ON MICROSATELLITE DNA POLYMORPHISMS.

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ABSTRACT

Genetic relationship among Nigeria's breeds of sheep and their crosses was studied using microsatellite DNA polymorphisms. DNA samples extracted from four Nigeria's breeds of sheep (West African Dwarf, the Balami, Uda and Yankassa) and their crosses were analysed using 30 microsatellite markers for diversity studies. Twenty of the markers were amplified by the sheep genome. Nineteen of the loci were polymorphic and were used to calculate genetic distances (Ds) between the breeds based on allele frequencies of the microsatellite. The phylogenetic relationships between the breeds were similarly estimated.

With the total number of loci studied, 285 alleles were generated and a high degree of heterozygosity was recorded, (0.57 to 0.72). a relatively high degree of reliability could be placed on the tree topology with the relationship between breeds portraying a closer relationship between the yankassa and Uda (Ds 0.356). The genetic distance (Ds) was 0.432, 0.534 and 0.665 between the West African Dwarf and the Yankassa, Uda and Balami respectively also indicating a closer relationship between the Yankassa and the WAD compared to the basically Northern breeds (Uda and Balami). This further confirms the evolutionary divergence of the breeds which makes them distinct entities.

The data suggests that microsatellite DNA markers are very useful tools for studying the genetic relationships among these sheep breeds. The highly polymorphic alleles could thus be exploited in breed improvement and development.

Keywords: Sheep, Microsatellite DNA, Polymorphisms, Genetic Distance.

INTRODUCTION

The domestic sheep (*Ovis aries*) during the last 10,000 years has attained a relatively high

increase in body size, a decrease in horn size and a change from a hairy moultly fleece to a white woolly fleece (Ryder 1983). Hundreds of local breeds and strains have been developed for different production systems throughout the world.

Further genetic improvement has occurred in the last 50 years from the application of quantitative genetics and selective breeding. In addition, studies of the physiology of the sheep have contributed to improved agricultural production and provided the sheep as useful models in the study of mammalian reproduction and neurology (Crawford *et al* 1995). Many economically important traits in sheep such as growth rate, body composition, disease resistance are multigenic in nature. If we are to understand the genetics of these complex traits, we need to know and exploit the wide genetic diversity that is present in the domestic sheep. Mapping of highly informative markers covering the majority of the sheep genome (Lander and Bostein 1989) and the utilisation of highly polymorphic microsaellite markers (Litt and Luty 1989, Tautz 1989) are the first essential steps.

Microsatellites are highly polymorphic two to five nucleotide repeat sequences, (for example, CA, GT) repeats that are widely dispersed in the mammalian genome. They have been shown to be powerful tools in genome mapping of man and his animals (Cornall *et al* 1991, Estoup *et al* 1993)

With over 14 million relatively unselected sheep population in Nigeria, widely dispersed all over the country, yet highly localised in their existence and adaptation, the need to characterise and exploit these diverse genotypes can not be overemphasized. Nigeria has basically four definitive breeds of sheep - the West African Dwarf (WAD), the Uda (UD),

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Balami (BAL) and the Yankassa (YANK). These are all adapted to different ecological niches except for the Yankassa which is widely spread across the country.

It is the aim of this study to utilize microsatellite DNA markers to genotype these sheep breeds and to determine their allelic differences and genetic relationship for future exploitation of their genes in breed improvement and development.

MATERIALS AND METHODS

Animals

7 mls blood samples were collected from the jugular vein of 60 unrelated sheep from Ogun, Oyo, Plateau and Kaduna States of Nigeria comprising the Uda (6), WAD (6), Balami (4) Yankassa (38) Uda X Yankassa cross (4) and an exotic breed the Merino (ME) (2). The buffy coat was extracted from these blood samples and was transported in 2 mls Urea/Tris/EDTA solution, to Roslin Institute, Scotland, for the DNA extraction and analyses. The DNA was extracted using the Phenol/Chloroform extraction method with the DNA dissolved in 500ul of sterile distilled water

and stored at 4°C before analyses.

Genetic Loci

30 microsatellite markers, that are widely distributed across the cattle genome, represented on the International Panel of Markers were used to screen the DNA samples. 20 of these markers amplified the sheep genome. Table 1 shows the primer sequences, chromosomal location and accession numbers in the cattle genome database.

PCR Conditions

The PCR analysis was carried out using fluorescently labelled PCR primers with 10 pM of each primer on 50ng of each DNA sample. The 10ul reaction mixture was made up of 1ul each of PCR buffer (Amersham) dCTP, DGTP, DTTP and dATP 20M each, Taq Polymerase 0.5 units per reaction and MgCl (1.5 - 3.0) depending on the primer. The mixture was cycled on the Hybaid Omnigene thermal cycler. Cycling conditions were, 3 mins at 93.5°C followed by 30 cycles at 94°C, 30 secs at annealing temperatures of 55 - 65°C and 30 secs elongation at 72°C. The last elongation step was prolonged to 9 mins.

Half of the PCR product was loaded with 5ul formamide dye before running of 1.5% agarose at 100 volts for 1 hr alongside a standard marker -

TABLE 2: SHEEP PCR MULTIPLEXES

	MARKER	CHR DYE	PCR DILN	Ta	Oc	Mg	ALLELE SIZE ALL.	NO
MULTIPLEX 1	ILSTS 5	10 TET	1:05	55	3		189-204	16
	INRA 35	16 TET	1:05	55	3		106-122	10
	BM1818	23 FAM	1:05	55	2		261-279	11
	ETH10-2	5 FAM	1:10	60	2		206-214	9
	ETH225	9 FAM	1:03	65	2		132-154	17
MULTIPLEX 2	HEL 1	15 TET	1:05	55	2		107-157	24
	CSSM66	14TET	1:10	60	2		173-213	22
	INRA37	11 FAM	1:05	55	2		109-141	18
MULTIPLEX 3	ETH 152	5 FAM	1:02	65	1.5		190-205	11
	MM 12	9 HEX	1:10	60	2		83-102	11
	TGLA 122	21 HEX	1:10	60	2		135-158	15
MULTIPLEX 4	SPS 115	15 TET	1:02	65	2		234-268	9
	BM2 113	2 FAM	1:02	60	3		124-160	16
	CSRM 60	10 HEX	1:10	60	3		77-95	10
MULTIPLEX 5	ETH 3	19 FAM	1:10	55	2		96-119	17
	BM 1824	1 TET	1:10	55	3		162-183	12
	TGLA 53	16 TET	1:10	55	2		147-167	20
	HEL 9	8 FAM	1:05	60	2		94-104	3
MULTIPLEX 6	INRA 63	18 HEX	1:05	55	2		162-182	12
MULTIPLEX 7	TGLA 126	20 HEX	1:02	55	2		113-134	14

Ta = annealing temperature
 All NO = number of alleles generated
 Mg = magnesium chloride concentration

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TABLE3: ALLELIC DIFFERENCES IN 20 MARKER LOCI OF NIGERIA'S SHEEP BREEDS

MARKER	CHR	YANKASSA NO RANGE		BALAMI NO RANGE		UDA NO RANGE		WAD NO RANGE		MERINO NO RANGE		UDA/YANKCROSS NO RANGE	
ETH225	9	14	127-154	4	142-147	9	142-154	6	125-149	2	146-148	7	143-150
INRA35	16	7	115-122	2	115-118	2	106-115	2	115-120	2	115-117	3	114-117
ILSTS5	10	15	189-204	5	191-203	8	191-204	7	191-195	4	190-198	6	190-201
ETH152	5	9	190-205	3	191-204	7	191-205	2	191-195	3	191-195	3	191-195
ETH10-2	5	9	206-214	5	207-212	6	206-211	5	206-211	4	206-212	4	206-209
INRA63	18	9	162-176	2	168-176	6	165-176	9	162-176	4	164-176	6	164-182
HEL9	8	2	102-103	2	102-103	3	94-103	1	102-102	1	102-102	2	102-103
HEL1	15	19	107-157	5	109-118	6	110-120	5	111-122	3	111-116	5	110-121
CSSM66	14	22	181-210	3	181-199	7	181-203	7	181-201	4	181-199	4	193-201
MM12	9	10	83-102	2	91-96	3	91-98	5	83-98	2	91-98	2	91-98
ETH3	19	15	96-111	5	96-107	8	98-112	6	99-107	3	105-107	7	99-119
BM2113	2	14	124-160	2	138-141	6	135-160	4	138-150	2	146-153	6	124-150
BM1824	1	10	170-183	7	161-178	6	171-178	5	172-177	3	172-175	4	172-178
CSRM60	10	7	78-87	3	82-84	4	78-84	3	82-84	4	82-95	4	77-84
TGLA122	21	9	136-146	5	136-145	6	136-158	3	136-143	2	136-137	4	135-156
SPS115	15	8	234-251	4	237-251	4	236-250	3	237-250	4	249-268	3	237-250
TGLA126	20	13	113-133	5	113-134	8	113-126	6	113-126	3	113-118	4	114-126
BM1818	23	11	261-279	3	261-265	5	261-271	5	261-269	3	261-265	4	261-271
INRA37	11	14	109-141	5	123-136	6	120-133	7	109-133	2	126-136	7	120-136
TGLA53	16	14	144-167	6	147-160	7	158-166	10	147-164	2	152-163	5	160-165
Total allele generated		222		78		117		101		57		90	
Average No per locus		11.1		3.9		5.85		5.1		2.9		5	

BR322 having allele size of 50 - 350 bps. The gel was viewed under semi-automated UV light to determine the dilution ratio and marker allele size.

Multiplexing and size characterisation were carried out with the other half of the PCR product after dilution (Table 2). 1ul of the diluted multiplexed product was loaded with 3ul of Internal size standard (TAMRA), run on 6% polyacrylamide denaturing gel for 3 - 3 1/2 hours and sequenced using the Applied Biosystems 373A Gene Sequencer (Perking Elmer), after denaturing at 90°C for 4 mins and rapid cooling in ice. Gel analyses was performed with Genescan 672 (Version 1.2) software. Allele frequencies were determined by direct counting using the Excel format. Frequency distribution per locus per breed was compared using SAS 1989 package. Genetic distance (Ds) was calculated using Nei's 1972 procedure as follows,

Nei's 1972 standard genetic distance (Ds):

$$Ds = \ln [J_{xy} / \sqrt{J_x J_y}]$$

$$\text{where } J_x = \sum_j \sum_i^{m_j} x_{ij}^2 / r \text{ and } J_y = \sum_j \sum_i^{m_j} y_{ij}^2 / r$$

are the average homozygosities over loci in population x and y respectively

$$J_{xy} = \sum_j \sum_i^{m_j} \frac{X_{ij} Y_{ij}}{r} \quad X \text{ and } Y; X_{ij} \text{ and } Y_{ij} \text{ being}$$

the frequencies of the m_j allele at the j th locus in population X and Y respectively, m_j is the number of alleles at the j th locus and r is the number of loci examined. The distance, neighbor-joining trees (UPGMA method version 3.57) and bootstrap values were generated with programmes in the Phylip package (Felsenstein 1993). Bootstrap values were compared over 100 replicates for trees by resampling loci.

RESULTS AND DISCUSSIONS

All loci sampled were found to be polymorphic in the sheep breeds except Hel 9 which is monomorphic in most breeds. The only alleles found in all the breeds being 102/103 which might probably be one and the same allele in the Yankassa, Balami, and the uda X Yankassa

TABLE 4: MEAN NUMBER OF MARKER ALLELES FOR CATTLE AND SHEEP BREEDS

Cattle Breeds	ND	BU	NDBU	GUD	WAR	HF	HBUF1	HBUF2	HVAR
No	76	63	6	6	16	20	39	20	3
Allele No	14.77	10.73	3.77	4.06	8.23	7.73	10.92	8.42	3.69
Heterozyg	0.53	0.62	0.50	0.62	0.59	0.61	0.66	0.64	0.70

Sheep Breeds	YANK	BAL	UDA	WAD	ME	UDYANK
No	38	4	6	6	2	4
Allele No	11.5	3.9	5.85	5.05	2.85	0.6
Heterozyg	0.57	0.61	0.68	0.58	0.72	0.65

TABLE 5: GENETIC DISTANCES BETWEEN NIGERIA SHEEP BREEDS AND CROSSES

YANKASSA						
BALAMI	0.4761					
BALAMI	0.3561	0.4335				
WEST AFRICAN DWARF	0.4318	0.6652	0.5343			
MERINO	0.5597	0.8047	0.7425	0.7136		
UDA X YANKASSA CROSSES	0.3874	0.6126	0.3491	0.661	0.5321	

cross. Allele 102 alone was found in the Merino and the WAD breeds whilst the Uda is the only breed in which both alleles 94 and 102 were found. a total of 285 alleles were generated ranging from 3 in Hel 9 to 24 in Hel 1 (Table 3), a large percentage of which showed very high degree of variation. The allelic mean per breed varied from 2.85 in the Merino to 11.55 in the Yankassa (Table 4), with average heterozygosities of 0.57 to 0.72.

From the matrices of genetic distance (Table 5), population were clustered by establishing the NJ (neighbor-joining method) dendograms. The dendograms based on Ds (Fig. 1), showed that the breeds were clearly separated from each other. These native breeds stood out as 3 distinct groups on the evolutionary scale. The Uda and Balami which are basically Northern breeds are clustered together with 65% occurrence bootstrap value far away from the WAD whilst the Yankassa stood in between the extremes of the West African Dwarf (a Southern humid zone breed) and the Uda and Balami (Northern arid Zone breeds). The Merino, an exotic breed, clustered with the Uda X Yankassa cross (73% occurrence) indicating a probably closer association between the two breeds despite the fact that the Uda cross is a Nigerian breed whilst the Merino is exotic.

Whilst genetic distances of 0.36 and 0.43 was found between the Uda and Yankassa and the

Uda and Balami respectively, distances of 0.66 was found between the WAD and the Balami as well as 0.53 between the WAD and the Uda and as high as 0.71 to 0.80 between the Merino and these 3 breeds showing that they might have diverged relatively early during evolution (Table 5). The efficiency of construction of phylogenetic trees by means of genetic distance is found to depend on linear relationships with time and the sampling error of the distance measures used (Nei et al 1983, Goldstein and Pollock 1994, Tajima and Takezaki 1994). The heterozygosity of 0.57 to 0.72 recorded in this study supports a very high heterozygosity for classical genetic markers (Nei 1987). In the case of microsatellite loci, the average heterozygosity seems to be generally between 0.5 and 0.8 (Bowcock et al, 1994, Forbes et al, 1995 Paetkau et al, 1995). They reported that heterozygosities, theoretically, is a function of an effective population size (N) and a mutation rate (V) per generation and that phylogenetic trees constructed with small number of loci for example less than 10 are not always reliable. Here we were lucky to have found 20 amplified loci in these breeds. The data therefore generated could be considered reliable enough to portray the relative degree of divergence within this population.

Moreover polymorphic MS loci in some species

Table 1 Primer sequences, accession numbers and allele sizes found in Nigeria's cattle and sheep

S/N	MARKER	CHR	DYE	PRIMER SEQUENCES	SPECIES	ALL SIZE	ALL NO	Ta	Mg	PCR Dn
1	ETH 225 (D9S1)	9	FAM	GATCACCTTGCCACTATTTCTCT ACATGACAGCCAGCTGCTACT	CAT (SH)	139-181 132-154	20 17	65 65	2 2	1:10 1:05
2	INRA 35 (D16S11)	16	TET	ATCCTTTGCAGCCTCCACATTG TTGTGCTTTATGACACTATCCG	CAT (SH)	104-127 106-122	17 10	55 55	3 3	1:02 1:05
3	ILSTS 5 (D10S25)	10	TET	GGAAGCAATGAAATCTATAGCC TGTCTGTGAGTTTGTAAAGC	CAT (SH)	181-196 189-204	12 16	55 55	3 3	1:10 1:05
4	ETH 152 (D5S1)	5	FAM	TACTCGTAGGGCAGGCTGCCTG GAGACCTCAGGTTGGTGATCAG	CAT (SH)	179-205 190-205	16 11	65 65	1.5 1.5	1:05 1:02
5	ETH 10-2 (D5S3)	5	FAM	GTTCAGGACTGGCCCTGCTAACA CCTCCAGCCCACTTTCTCTCTC	CAT (SH)	199-226 206-214	19 9	60 60	2.5 2	1:20 1:10
6	INRA 63 (D18S5)	18	HEX	ATTTGCACAAGCTAAATCTAACCC AAACCACAGAAATGCTTGGAAAG	CAT (SH)	176-197 162-182	17 12	60 60	1.5 3	1:02 1:05
7	INRA 5-2 (D12S4)	12	HEX	CAATCTGCATGAAGTATAAATAT CTTCAGGCATACCCCTACACC	CAT (SH)	110-179	31	60	2.5	1:05
8	HEL 9 (D8S4)	8	FAM	CCCATTGCTCTTCAGAGGT CACATCCATGTTCTCACCAC	CAT (SH)	149-171 94-108	21 4	60 60	1.5 2	1:05 1:03
9	HEL 1 (D15S10)	15	TET	CAACAGCTATTTAACAAGGA AGGCTACAGTCCATGGGATT	CAT (SH)	101-125 107-157	22 24	50 55	1.5 2	1:20 1:05
10	CSSM 66 (D14S31)	14	TET	ACACAAATCCTTTCTGCCAGCTGA AATTTAATGCACTGAGGAGCTTGG	CAT (SH)	179-200 173-213	20 22	60 60	2 2	1:10 1:10
11	Mm 12 (D9S20)	9	HEX	CAAGACAGGTGTTTCAATCT ATCGACTCTGGGGATGATGT	CAT (SH)	105-164 83-102	26 11	60 60	2 2	1:05 1:05
12	ETH 3 (D19S2)	19	FAM	GAACCTGCCTCTCCTGCATTGG ACTCTGCCTGTGGCCAAAGTAGG	CAT (SH)	103-131 96-119	21 17	60 55	1 2	1:10 1:05
13	BM 2113 (D2S26)	2	FAM	GCTGCCTTTACCAAAATACCC CTTCCTGAGAGAAGCAACACC	CAT (SH)	124-147 124-160	16 16	60 60	1.5 3	1:05 1:02
14	BM 1824 (D1S34)	1	TET	GAGCAAGGTGTTTTTCCAATC CATTTCTCCAACCTGCTTCCTTG	CAT (SH)	180-199 162-183	12 12	60 55	3 3	1:05 1:10
15	CSRM 60 (D10S5)	10	HEX	AAGATGTGATCCAAGAGAGAGGCA AGGACCAGATCGTGAAAGGCATAG	CAT (SH)	91-108 77-95	15 10	60 60	1.5 3	1:05 1:10
16	ILSTS 6 (D7S8)	7	FAM	TGTCTGTATTTCTGCTGTGG ACACGGAAGCGATCTAAACG	CAT (SH)	285-306	17	60	2	1:05
17	TGLA 122 (D21S6)	21	HEX	CCCTCCTCCAGGTAATCAGC AATCACATGGCAAATAAGTACATAC	CAT (SH)	134-167 135-158	20 15	60 60	2 2	1:05 1:05
18	HEL 13 (D11S15)	11	TET	TAAGGACTTGAGATAAGGAG CCATCTACCTCCATCTTAAC	CAT (SH)	184-196	10	50	1.5	1:20
19	SPS 115 (D15)	15	FAM	AAAGTGACACAACAGCTTCTCCAG AACGAGTGTCTAGTTTGGCTGTG	CAT (SH)	244-260 234-268	14 9	65 65	2 2	1:02 1:02
20	TGLA 126 (D20S1)	20	HEX	CTAATTTAGAATGAGAGAGGCTTCT TTGGTCTCTATTCTCTGAATATTCC	CAT (SH)	112-128 113-134	15 14	55 60	2 2	1:10 1:02
21	TGLA 226	18	TET	AGTGGAAATCCAGATAAGATGTATCA ACATGAAAAGAAGCAATATCGTAAC	CAT (SH)	126-158	18	55	1	1:10
22	HAUT 24 (D22S26)	22	TET	CTCTCTGCCTTTGTCCCTGT AATACACTTTAGGAGAAAAATA	CAT (SH)	100-134	34	55	3	1:02
23	BM 1818 (D23S21)	23	TET	AGCTGGGAATATAACCAAGG AGTGCTTTCAAGGTCCATGC	CAT (SH)	209-275 261-279	24 11	55 55	2 2	1:20 1:05
24	TGLA 53 (D16S3)	16	TET	GCTTTCAGAAATAGTTTGCAATCA ATCTTCACATGATATTACAGCAGA	CAT (SH)	143-186 147-167	34 20	55 55	1.5 3	1:20 1:10
25	INRA 37 (D10S12)	11	FAM	GATCCTGCTTATATTTAACAC AAAATTCATGGAGAGAGAAAC	CAT (SH)	100-140 109-141	29 18	55 55	2 2	1:05 1:05

Figure 1: Phenogram of Nigerian sheep breeds

have been reported to be monomorphic in others and even in breeds within the same species. This same situation was discovered with Hel 9 which is polymorphic in cattle but found to be monomorphic in these sheep breeds except in the Uda breed. The reason for the difference is still obscure. Despite the small numbers of animals used per breed and overall loci considered, the use of these MS loci demonstrated the ease with which polymorphism between breeds and between species could be examined. They have similarly proved very useful in studying the relationship of breeds within these Nigerian sheep population.

The divergence established through the dendrogram showed the Uda and Balami which are basically Northern breeds been 0.534 and 0.665 gene distance away respectively from the West African Dwarf which is a Southern rain forest zone breed. Microsatellites are genes that generally reveal a large number of different alleles that are selectively neutral (i.e. genes that do not confer on the individual a selective advantage over others). The extent of divergence in allele frequencies for such selectively neutral loci is therefore considered as a function of the level of isolation of one population from another (Takezaki and Nei 1996). Hence the microsatellite is preferred as a tool to study divergence. Since genetic diversity results from a particular historic development of the populations under consideration, the biological interpretation of the genetic distance depends largely on the divergence model used and the notion that the alleles are identical by descent. Genetic distance and tree construction methods give insight into the genetic uniqueness of breeds under investigation (Thaon d'Arnoldi et al, 1998), hence from the NJ-UPGMA method thus utilised the West African Dwarf with Ds values of 0.66 and 0.53 to Balami and Uda breeds respectively and 0.71 to the Merino cannot be said to be close when compared to the values of 0.43 to the Yankassa or 0.36 between the

Yankassa and the Uda. This therefore goes to proffer some explanation as to why these breeds have persistently been difficult to raise outside their zone of origin.

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REFERENCES

- BOWCOOK, A.M., A. RUTZ-LINARES, J. TAMFAHRDE, E. MINCHAND J.R. KIDD (1994). High resolution of human evolutionary trees with polymorphic microsatellites. *Nature* 368: 455 - 457.
- CORNALL, R.J., T.J. AITMAN, C.M., HEARNE and J.A. TODD (1991). The generation of a library of PCR analyses microsatellite variants for genetic mapping of the mouse genome. *Genomics* 10: 874 - 881.
- CRAWFORD, A.M., K.G. DODDS, A.J. EDE., C.A. PIERSON, G.W. MONTGOMERY, H.G. GARMONSWAY, A.E. BEATTIE, K. DAVIES, J.F. MADDOX (1995). An autosomal genetic Linkage map of the sheep genome. *Genetics* 140: 703 - 724.
- ESTOUP, A., A. PIESA, F. KRIEG, D. VAIMAN, and R. GUYPMARD (1993). (CT)_n and (GT)_n microsatellites: a new class of genetic markers for *salmo trutta* L. (brown trout). *Heredity* 71: 488 - 496.
- FORBES S.H., H.T. HONG, F.C. BUCHANAN, A.M., CRAWFORD and F.W. ALLENDORF (1995). Microsatellite evolution in congeneric mammals, domestic and bighorn sheep. *Mol. Biol. Evol.* 12: 1106 - 1113.
- GOLDSTEIN, D.B. and D.D. POLLOCK (1994). Least Squares estimation of Molecular distance noise abatement in phylogenetic reconstruction. *Theor. Pop. Biol.* 45: 219 - 226.
- LANDER, E.S. and BOTSTAN (1989). Mapping Mendelian factors underlying quantitative traits using RFLP Linkage maps. *Genetics* 121: 185 - 199.
- LIT M. and J.A. LUTTY (1989). A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *Ame. J. Hum. Genet* 44: 397 - 401.
- NEI M. (1972). Genetic distance between

- populations. *Ame. nat.* 106: 283 - 291.
- NEI M. (1987). *Molecular evolutionary genetics*
Columbia University Press New York.
- NEI M., F. TAJIMA and Y. TATENO (1983).
Accuracy of estimated phylogenetic trees from
molecular data. *J. Mol. Evol.* 19: 153 - 170.
- PAETKAU D., W. CALVERT, I. STIRLING and C.
STROBELLE (1995). Microsatellite analysis of
population structure in Canadian polar bears. *Mol.*
Ecol. 4: 347 - 354.
- RYDER M.L. (1983). *Sheep and Man.* duckworth
Press, London.
- SATOU N. and NEI (1987). The neighbor-joining
method: a new method for reconstructing
phylogenetic trees. *Mol. Biol. Evol.* 4: 406 - 425.
- TAJIMA, F. and N. TAKEZAKI (1994). Estimation
of evolutionary distance for reconstructing molecular
phylogenetic trees. *Mol. Biol. Evol.* 11: 278 - 286.
- TAKEZAKI N. and M. NEI (1996). Genetic distance
and reconstruction of phylogenetic trees from
microsatellite DNA. *Genetics* 144: 389 - 399.
- TAUTZ, D. (1989). Hypervariability of simple
sequences as general source for polymorphic DNA
markers. *Nucleic acid Res.* 17: 6463 - 6471.
- THAON D'ARNOLDI D., J.L. FAULLEY and L.
OLLIVIER (1998). an overview of the Weitzman
approach to diversity. *Genetics, Selection and*
Evolution 30: 149 - 161.