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Estimation of genetic diversity between three Saudi sheep breeds using DNA markers

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The genetic variation of Najdi, Harri and Awassi breeds of Saudi sheep prevailing in Raniah province of Makka district were assessed and compared to Sudanese Desert sheep using random amplified polymorphic DNA polymerase cahin reaction (RAPD-PCR) technique. Five primers successfully amplified distinguishable 40 bands with an average of 96% polymorphism revealing that Saudi sheep breeds possess the needed genetic variation required for further genetic improvement. The resulted dendrogram showed that, there are two main separate clades. The Desert sheep is genetically distant and appeared as out-group from the Saudi sheep breeds. The first main clade included all of the Najdi individuals and only two individuals from Harri breed. While, the second main clade comprised two subgroups, the first one included individuals from Harri breed and the second included both Harri and Awassi individuals. The cluster analysis shows that Najdi breed is genetically different from both Harri and Awassi and that some Harri individuals showed genetic closeness to Awassi. The present study will help to clarify the image of the genetic diversity of these local Saudi sheep breeds in Raniah province and should be followed by further studies using advanced DNA markers and all available breeds in the kingdom to get the precise estimation of the phylogeny of these local genetic resources.

Key words: Dendrogram, biodiversity, Sudanese sheep, random amplified polymorphic DNA (RAPD), Saudi sheep.

INTRODUCTION

The population of sheep in the Kingdom of Saudi Arabia is about 5.2 million head (Saudi Ministry of Agriculture, 2011). In Raniah Province of Makka district alone, there are 250000 heads of sheep (Al Faraj, 2003). Harri and Najdi sheep breeds are Saudi local reflect good adaptive traits to the local environmental conditions and meet the

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Abbreviations: RAPD, Random amplified polymorphic DNA; **PCR,** polymerase chain reaction.

Saudi consumer needs. Najdi is the principal native sheep breed in the eastern province of Saudi Arabia (Aljumaah et al., 2014). Awassi (also known as Naemi), sheep have been exported from its origin in east of the Mediterranean to more than 30 countries in all continents of the world including KSA (Galal et al., 2008). It is known for its good milk production (Al-Atiyat and Aljumaah, 2014). Indigenous sheep breeds are valuable source of genetic material due to adaptation to local harsh environmental conditions, nutritional fluctuations and resistances to diseases and parasites (Nsoso et al., 2004; Galal et al., 2008). Unfortunately, accelerated decline of biodiversity worldwide was reported and 20% of the domestic animal breeds are at risk of extinction (FAO, 2000; Kunene et al., 2009). Particularly for sheep, it is estimated that 180 sheep breeds (14%) are extinct (Cardellino, 2004; FAO, 2007). There is terrible risk that most breed may perish before they have been exclusively recognized and exploited. Conservation and maintenance of animal genetic biodiversity of local breeds will facilitate the effective management of farm animal genetic resources. There is a need to genetically re-evaluate these breeds to assess the existing population structure and differences which would serve to facilitate the future conservation programs. On the basis of microsatellite data, considerable genetic differentiation was recently reported in Saudi Najdi sheep (Musthafa et al., 2012). The first step in the conservation and utilization of indigenous sheep breeds is characterization and evaluation of genetic diversity which is a prerequisite for improving any species (FAO, 2007; Bjornstad and Roed, 2001; Notter, 1999).

The traditional phenotypic characterization can now be complemented by molecular markers and sophisticated statistical techniques for data analysis. Random amplified polymorphic DNA (RAPD) is a polymerase chain reaction (PCR) based fingerprinting technique that amplifies random DNA fragments with single short primers of arbitrary nucleotide sequence under low annealing stringency (Williams et al., 1999; Awad et al., 2010). The RAPD markers have been described as a simple and easy method to use for estimation of genetic variability among breeds or species (Kumar et al., 2008; Ruane, 2000). The objective of the present study was to utilize the RAPD technique to characterize three Saudi sheep breeds (namely Awassi, Harri and Najdi) in Raniah province and to estimate the genetic diversity within and between these breeds and Sudanese Desert Sheep as outbreed.

MATERIALS AND METHODS

Study area

The study area pertains to Raniah Province of Makkah district (12° 30 N, 42° E) in the west part of Saudi Arabia extending over 62,000 km^2. Raniah Province lies about 870 km south- west to Riyadh, 380 km west to Taif and 150 km north to Bishah. The province has similar meteorological and ecological attributes with the rest of the Arabian Peninsula. It is characterized by hot arid desert type climate, with average annual rainfalls of 90 mm, maximum temperature between 34 and 45°C in summer and between zero and 20°C in winter with an average relative humidity of 22% (Al Faraj, 2003).

Animals

Full mouthed unrelated females were randomly selected from three Saudi sheep breeds, namely, Awassi (Naeimi), Harri and Najdi to serve as blood donors. Twenty individuals were sampled from Najdi, 14 from Harri and five from Awassi. Blood was also collected from three Sudanese desert sheep to serve as outbreed for comparison.

Genomic DNA extraction

Blood samples from Jugular vein were collected from full mouthed unrelated female. At least 5 ml blood sample was drawn from the vein in the neck of each animal and collected in EDTA vacutainers. The blood was gently mixed with anticoagulant and kept at -20°C. Genomic DNA was extracted from peripheral blood lymphocytes according to instructions of blood DNA preparation kit (Jena Bioscience, Germany).

PCR amplification

The PCR amplification was performed in a 25 µl reaction volume, using Promega PCR master mix according to the instructions by the manufacturer with 30 Pmol from each of the primers: Initial denaturation at 94°C for 2 min, followed by 35 cycles consisting of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 2 min and a final extension at 72°C for 2 min. Amplified products were electrophoresed on 1.5% agarose gel at constant voltage and 1X TBE for approximately 1.5 h. They were visualized by staining with ethidium bromide and photographed under ultraviolet light and molecular weights were estimated using 1 Kbp DNA ladder.

Scoring and statistical analysis

PCR products were scored across the lanes as variables. The presence of a band of amplified DNA was scored as (1) and absence as (0). The data generated was used for calculation of similarity matrix based on Nei and Li (1979). Very faint bands were excluded from the analysis. Similarity coefficients were utilized to generate a phylogenetic tree (dendrogram). Pairwise genetic distance between individuals were calculated by the percentage disagreement method. These data were used in cluster analysis with the unweighted pair-group method using arithmetic averages (UPGMA), in which samples were grouped based on their similarity with the aid of statistical software package STATISTCA- version 9 (StatSoft Inc., 2009).

RESULTS AND DISCUSSION

Information on genetic relationships in livestock within and between species has several important applications for genetic improvement and breeding programmes (Appa Rao et al., 1996). Comprehensive knowledge of the existing genetic variability is the first step for the

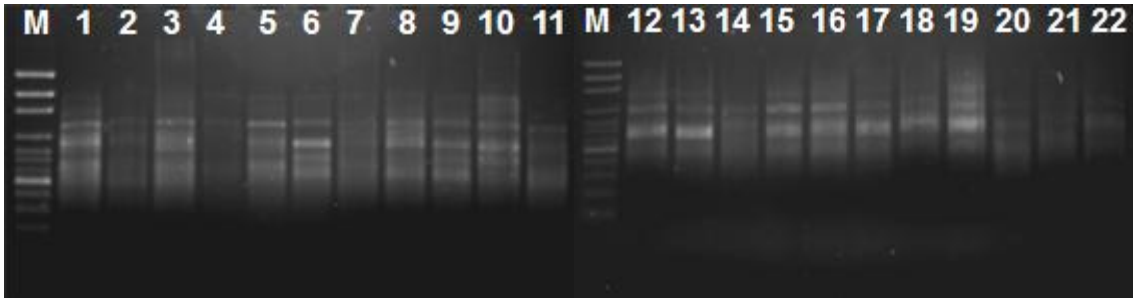

Figure 1. RAPD fingerprint generated from Najdi (1-20) and Harri (21-22) sheep breeds using OPL20 primer.

Table 1. The sequences of primers used and their polymorphic bands among three Saudi and one Sudanese sheep breeds.

Primer	Sequence of primer (5'-3')	Total number of bands	Number of polymorphic bands	Number of monomorphic bands	Polymorphism (%)
OPL-11	ACGATGAGCC	7	7	0	100
OPAL-20	AGGAGTCGGA	9	9	0	100
OPAL-15	AGGGGACACC	9	9	0	100
OPE-18	GGACTGCAGA	9	9	0	100
OPB-5	TGCGCCCTTC	6	5	1	83.33
Total		40	39	1	
Average		8	7.8	0.2	96.67

RAPD primers from Operon Technologies Inc. (Alameda Calif., USA).

conservation and exploitation of domestic animal biodiversity. Therefore, the objective of this study was to evaluate the genetic diversity of sheep breed in Raniah province of Saudi Arabia based on RAPD analysis. Five, out of 17 tested primers successfully amplified polymorphic bands between the different sheep breeds. The amplified PCR product of DNA showed identical band patterns with similar intensity (Figure 1). Out of the total distinguished 40 amplified fragments, 39 were polymorphic with an average of 7.8 bands. The maximum number of fragments (9 bands) was produced by three primers with 100% polymorphism, while the minimum numbers of fragments were produced by primer OPB-5 with 83.33% polymorphism (Table 1). The very high polymorphic rate (96.67%) indicated that the studied sheep breed possess the needed genetic variation for potential future preservation and breed development. Although, all studied population of Saudi Najdi, Hbsi, Arb, and Naemi sheep had substantial levels of genetic variation, but Najdi sheep had the highest gene diversity (Aljumaah et al., 2014).

Table 2 shows the genetic distance between individuals of the four goat breeds. Individuals designated with numbers from 1 to 20 are Najdi, from 21 to 34 are Harri, from 35 to 39 are Awassi and from 40 to 42 are Sudanese Desert Sheep. The highest genetic distance (0.53) was found between Najdi individual (N14) and the three Desert sheep individuals (Desert sheep-1-3). On the other hand, the least genetic distance (0.0) was found between Najdi individuals N1 and N2 and also between the two Desert sheep individuals (40 and 42). Genetic distance value of 0.0 reflects very high similarity between any two individuals. The distance measure between two clusters is calculated from the formula: *D=1-C*; where, D is the distance and C the correlation between object clusters. If objects are highly correlated, they will have a correlation value close to 1 and genetic distance value close to zero. Therefore, highly correlated clusters are nearer to the bottom of the dendrogram. Object clusters that are not correlated have a correlation value of zero and a corresponding genetic distance value of 1. Objects that are negatively correlated will have a correlation value of minus1 and genetic distance of 2.

As shown in Figure 2, the resulted dendrogram constructed from RAPD-PCR data showed that the Desert Sheep is genetically distant and appeared as out-group to the Saudi goat breeds. The result also shows that, there are two main separate clades. Most of the individuals that belong to the same breed were clustered together. The first main clade included Najdi individuals (N1-N20) and only two individuals from Harri breed. While the second main clade comprises two subgroups, in which the first subgroup contained only individuals from Harri breed (H3-H9 individuals). On the other hand, the second subgroup of the second main clade included Harri and Awassi individuals. The cluster analysis shows

Table 2. Matrix of RAPD dissimilarity among three Saudi sheep and Desert sheep breeds based on Nei and Lei coefficients

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	0.00																				
2	**0.00**	0.00																			
3	0.15	0.15	0.00																		
4	0.23	0.23	0.18	0.00																	
5	0.30	0.30	0.15	0.08	0.00																
6	0.30	0.30	0. 15	0.08	**0.00**	0.00															
7	0.38	0.38	0.23	0.15	0.08	0.08	0.00														
8	0.40	0.40	0.25	0.18	0.15	0.15	0.13	0.00													
9	0.30	0.30	0.20	0.13	0.15	0.15	0.18	0.15	0.00												
10	0.35	0.35	0.20	0.18	0.15	0.15	0.13	0.15	0.15	0.00											
11	0.33	0.33	0.23	0.15	0.18	0.18	0.20	0.18	0.13	0.13	0.00										
12	0.25	0.25	0.15	0.18	0.20	0.20	0.23	0.25	0.20	0.15	0.18	0.00									
13	0.28	0.28	0.28	0.25	0.28	0.28	0.25	0.33	0.23	0.23	0.25	0.18	0.00								
14	0.28	0.28	0.23	0.30	0.28	0.28	0.30	0.33	0.28	0.28	0.30	0.28	0.15	0.00							
15	0.38	0.38	0.38	0.35	0.38	0.38	0.30	0.38	0.33	0.38	0.35	0.33	0.20	0.15	0.00						
16	0.35	0.35	0.30	0.33	0.35	0.35	0.28	0.30	0.30	0.25	0.23	0.20	0.18	0.18	0.13	0.00					
17	0.40	0.40	0.30	0.33	0.30	0.30	0.28	0.35	0.35	0.30	0.33	0.25	0.18	0.23	0.23	0.15	0.00				
18	0.33	0.33	0.28	0.30	0.33	0.33	0.30	0.38	0.38	0.38	0.35	0.28	0.25	0.25	0.15	0.13	0.18	0.00			
19	0.40	0.40	0.40	0.28	0.30	0.30	0.28	0.35	0.35	0.35	0.33	0.35	0.28	0.33	0.23	0.20	0.20	0.18	0.00		
20	0.38	0.38	0.38	0.30	0.33	0.33	0.30	0.38	0.38	0.38	0.35	0.38	0.30	0.35	0.25	0.23	0.23	0.15	0.08	0.00	
21	0.38	0.38	0.38	0.25	0.28	0.28	0.20	0.28	0.28	0.28	0.25	0.33	0.25	0.30	0.20	0.18	0.23	0.20	0.13	0.10	0.00
22	0.33	0.33	0.33	0.15	0.23	0.23	0.20	0.23	0.23	0.28	0.30	0.28	0.25	0.30	0.25	0.23	0.23	0.20	0.18	0.20	0.15
23	0.38	0.38	0.43	0.30	0.38	0.38	0.30	0.38	0.33	0.33	0.35	0.38	0.30	0.35	0.35	0.33	0.33	0.40	0.33	0.40	0.30
24	0.30	0.30	0.35	0.23	0.30	0.30	0.23	0.30	0.25	0.25	0.28	0.30	0.23	0.28	0.28	0.25	0.30	0.33	0.25	0.28	0.18
25	0.40	0.40	0.35	0.33	0.35	0.35	0.28	0.35	0.30	0.30	0.33	0.30	0.23	0.33	0.33	0.30	0.30	0.28	0.35	0.33	0.28
26	0.40	0.40	0.35	0.28	0.30	0.30	0.23	0.25	0.30	0.30	0.33	0.35	0.23	0.33	0.28	0.25	0.30	0.23	0.25	0.23	0.23
27	0.38	0.38	0.38	0.30	0.38	0.38	0.30	0.38	0.33	0.33	0.35	0.38	0.25	0.30	0.30	0.28	0.28	0.30	0.33	0.30	0.30
28	0.48	0.48	0.48	0.30	0.38	0.38	0.30	0.38	0.38	0.38	0.35	0.43	0.30	0.35	0.25	0.28	0.28	0.30	0.23	0.30	0.25
29	0.48	0.48	0.48	0.30	0.38	0.38	0.30	0.38	0.38	0.38	0.35	0.43	0.30	0.35	0.25	0.28	0.28	0.30	0.23	0.30	0.25
30	0.35	0.35	0.40	0.23	0.30	0.30	0.28	0.25	0.30	0.35	0.28	0.40	0.23	0.28	0.23	0.25	0.30	0.28	0.25	0.28	0.23
31	0.35	0.35	0.45	0.28	0.35	0.35	0.43	0.35	0.35	0.45	0.43	0.45	0.33	0.38	0.43	0.45	0.40	0.43	0.35	0.43	0.43
32	0.33	0.33	0.28	0.10	0.18	0.18	0.25	0.28	0.23	0.28	0.20	0.28	0.30	0.35	0.40	0.38	0.33	0.35	0.33	0.35	0.35
33	0.33	0.33	0.28	0.10	0.18	0.18	0.25	0.28	0.23	0.28	0.25	0.28	0.30	0.35	0.40	0.38	0.33	0.35	0.28	0.35	0.35
34	0.33	0.33	0.33	0.15	0.23	0.23	0.25	0.33	0.28	0.28	0.30	0.28	0.30	0.40	0.40	0.38	0.38	0.35	0.28	0.35	0.35
35	0.30	0.30	0.25	0.18	0.25	0.25	0.23	0.30	0.30	0.30	0.33	0.25	0.33	0.38	0.33	0.30	0.35	0.23	0.25	0.28	0.33
36	0.40	0.40	0.35	0.18	0.25	0.25	0.23	0.20	0.25	0.30	0.33	0.30	0.38	0.48	0.43	0.40	0.40	0.38	0.30	0.38	0.33
37	0.40	0.40	0.35	0.18	0.25	0.25	0.23	0.25	0.25	0.30	0.33	0.30	0.38	0.48	0.43	0.40	0.40	0.33	0.30	0.38	0.33
38	0.33	0.33	0.33	0.20	0.28	0.28	0.25	0.33	0.33	0.33	0.35	0.33	0.40	0.50	0.45	0.43	0.43	0.35	0.33	0.35	0.35
39	0.35	0.35	0.30	0.18	0.25	0.25	0.28	0.35	0.30	0.30	0.33	0.30	0.33	0.38	0.38	0.35	0.35	0.28	0.25	0.28	0.33
40	0.35	0.35	0.40	0.28	0.35	0.35	0.33	0.40	0.40	0.40	0.38	0.35	0.38	**0.53**	0.43	0.40	0.35	0.33	0.25	0.18	0.23
41	0.40	0.40	0.45	0.33	0.40	0.40	0.38	0.45	0.45	0.45	0.43	0.40	0.38	**0.53**	0.43	0.40	0.35	0.33	0.25	0.18	0.28
42	0.35	0.35	0.40	0.28	0.35	0.35	0.33	0.40	0.40	0.40	0.38	0.35	0.38	**0.53**	0.43	0.40	0.35	0.33	0.25	0.18	0.23

Table 2. Contd.

22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
0.00																				
0.25	0.00																			
0.18	0.13	0.00																		
0.28	0.23	0.15	0.00																	
0.23	0.28	0.20	0.15	0.00																
0.25	0.15	0.13	0.13	0.13	0.00															
0.25	0.15	0.18	0.23	0.18	0.10	0.00														
0.25	0.15	0.18	0.23	0.18	0.10	0.00	0.00													
0.23	0.33	0.25	0.30	0.15	0.23	0.18	0.18	0.00												
0.28	0.28	0.35	0.40	0.25	0.28	0.23	0.23	0.20	0.00											
0.25	0.35	0.33	0.38	0.28	0.25	0.25	0.25	0.23	0.23	0.00										
0.25	0.30	0.33	0.38	0.28	0.25	0.20	0.20	0.23	0.18	0.05	0.00									
0.25	0.25	0.28	0.33	0.23	0.25	0.20	0.20	0.28	0.18	0.15	0.10	0.00								
0.23	0.33	0.30	0.30	0.20	0.23	0.23	0.23	0.25	0.30	0.18	0.13	0.13	0.00							
0.18	0.28	0.25	0.30	0.20	0.28	0.23	0.23	0.25	0.20	0.23	0.18	0.13	0.15	0.00						
0.18	0.28	0.25	0.25	0.25	0.28	0.23	0.23	0.30	0.25	0.23	0.18	0.13	0.15	0.05	0.00					
0.25	0.20	0.28	0.28	0.23	0.25	0.25	0.25	0.33	0.28	0.25	0.20	0.10	0.13	0.13	0.13	0.00				
0.23	0.38	0.35	0.35	0.25	0.28	0.28	0.28	0.30	0.30	0.18	0.13	0.13	0.10	0.20	0.20	0.18	0.00			
0.28	0.43	0.30	0.35	0.35	0.38	0.38	0.38	0.35	0.50	0.38	0.38	0.38	0.30	0.35	0.35	0.33	0.30	0.00		
0.33	0.48	0.35	0.40	0.35	0.38	0.38	0.38	0.35	0.50	0.38	0.38	0.38	0.30	0.40	0.40	0.38	0.30	0.05	0.00	
0.28	0.43	0.30	0.35	0.35	0.38	0.38	0.38	0.35	0.50	0.38	0.38	0.38	0.30	0.35	0.35	0.33	0.30	0.00	0.05	0.00

that Najdi breed is genetically different from both Harri and Awassi, with maximum distance from Desert sheep breed. Also, individuals from the same breed are genetically close to each other. However, some individuals from Harri (H11, H12, H13) showed genetic closeness to Awassi. The close kinship between Harri and Awassi might suggest some past crossing between these two geographically close populations.

In a study aimed to characterize genetic constitution of Awassi, Harri and Habsi Saudi sheep, using random amplified polymorphic DNA (RAPD) technique, the highest homogeneity was observed within Harri breed followed by Habsi and Awassi breeds (40 and 24.2%, respectively) (Sabir et al., 2013). The genetic structure of Saud sheep population including Najdi, Hbsi, Arb, and Naemi was investigated using microsatellite revealing substantial genetic variability, with average heterozygosity range of 0.759 to 0.811 (Aljumaah et al., 2014). The genetic characterization, however, should be a continuous process of surveying and monitoring of the existing indigenous breeds.

Conclusion and recommendation

The very high polymorphic rate (96.67%) indicated that the studied sheep breed possess the needed genetic variation for further potential future preservation and breed development. The result from this study shows that Najdi breed is genetically different from both Harri and Awassi and that some individuals from Harri showed genetic closeness to Awassi. The present study will help to clarify the image of the genetic diversity of these local Saudi sheep breeds and should be followed by further studies using large number of animals from different geographical regions in the kingdom to get the precise estimation of the phylogeny of these local genetic resources.

Conflict of interests

The authors did not declare any conflict of interest.

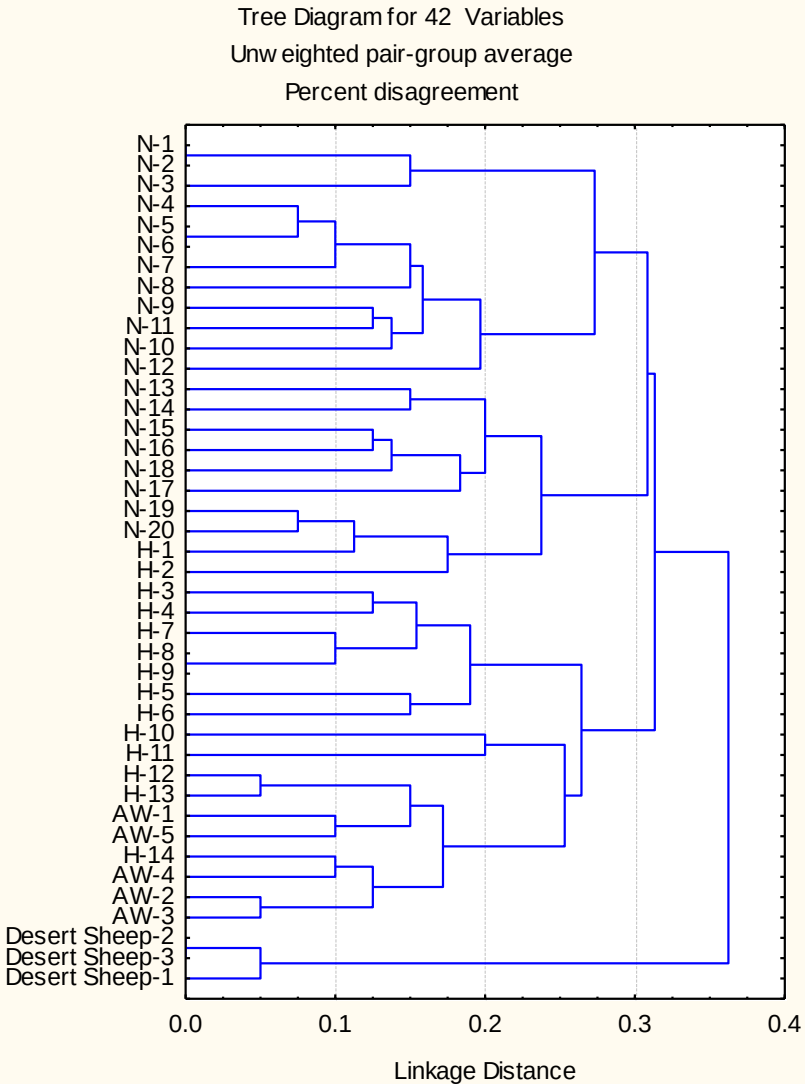

Figure 2. Phylogenic tree showing relationships among the four sheep breeds obtained by RAPD-PCR analysis using five primers. Individuals designated with N are Najdi; with H are Harri and with A are Awassi sheep breed.

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