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Molecular characterization of Hazaragie sheep native to Central Afghanistan

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ABSTRACT

Hazaragie is a small size, fat-tailed sheep breed found in the mountainous area of central Afghanistan. To characterize the breed on molecular level, DNA was extracted from blood samples of 44 animals randomly selected from their breeding tract, and each of 31 SSR markers was subjected to PCR using individual DNA sample, for amplification. Out of 31 SSR Markers 4 did not amplify, however, the remaining 27 were polymorphic giving total number of 170 alleles with average number of alleles 6.296 per locus, ranged from 3 (OarCP34) to 11 (BM1329). Mean effective number of alleles were 4.394 with allelic richness of 3.584 per locus. Higher heterozygosity (0.825) was observed in the population and hence expected heterozygosity (0.772), average heterozygosity (0.757) and gene diversity (0.772) were also higher. Inbreeding estimates within the population was calculated to be –0.069 on average across all loci. Most of the loci and overall population significantly deviate from Hardy Weinberg equilibrium. Polymorphism Information Content and Shannon information index was 0.534 and 1.581 respectively. Tests for Mutation drift equilibrium showed the absence of genetic bottleneck in the population. There was significant number of loci with heterozygosity excess under 2 of the 3 mutation models. Qualitative test utilizing allele frequencies showed the abundance of low frequency alleles and followed normal L-shape form on graph; represent the absence of genetic bottleneck in the population in recent past.

Key words: Genetic bottleneck, Genetic diversity, Hazaragie sheep, Molecular characterization, Simple sequence repeat (SSR) markers

The Hazaragie sheep is found in the mountainous area of central Afghanistan (from Kabul to Heart also called Hazarajat). Total population of the breed in Hazarajat is about 0.6 million (Rashiq 2004). The breed is mainly kept by the Hazara people of the area, who are responsible for its name and development. Hazaragie is a small sized mutton type fat-tailed breed, well adapted to the cold environmental condition of region (temperature, winter –15° and summer 0–26°C, snowfall 40cm/year) as reported by Peter and Blood (2001).

Genetic diversity studies can identify alleles that might affect the ability of the organism to survive in its existing habitat, or might enable it to survive in more diverse habitats. The intensity and standardization of animal production are threatening the existence of many domestic animal breeds (Scherf 2000). In establishing priorities for the conservation of breeds on the basis of genetic arguments, traits, genes and gene combinations should all be considered. In addition, attention should be paid to the uniqueness of individual features (Scherf 2000, Barker *et al.* 2001). It is not possible to know which traits and alleles are important in the future. This equals to the situation in natural populations, where it is not known beforehand that which geographic population initiates a speciation event or provides the crucial beneficial genotypes when the environment changes (Soysal *et al.* 2005).

Microsatellites have been considered as marker of choice in animal genetic studies because of their utility in providing valuable genetic information (Vignal *et al.* 2002, Baumung *et al*. 2004). These markers are used for genome characterization and diversity analysis in different livestock species (Mukesh *et al.* 2004, Soysal *et al.* 2005, Olowofeso *et al.* 2005, Pandey *et al.* 2006, Sodhi *et al.* 2006, Kumar *et al.* 2007, Kathiravan *et al.* 2009, Ibrahim *et al.* 2010, Mishra *et al.* 2009 and 2010, Vijh *et al.* 2010, Sharma *et al.* 2010 and Yadav *et al.* 2011). The current study is the first ever attempt to characterize the genome from Hazaragie sheep population. The objectives of the current study included estimation of genetic diversity, inbreeding and mutation drift equilibrium within the population.

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MATERIALS AND METHODS

Blood sampling for DNA isolation: Sheep (44) from purebred flocks of Hazaragie sheep, were selected randomly from distinct locations in their breeding tract, and was sampled for blood collection. Blood sample (3 ml) was collected from individuals using disposable syringe by puncturing the jugular vein and were transferred to the EDTA coated labeled vacutainers. Samples were stored at –20°C until DNA isolation.

Isolation of genomic DNA from blood: Genomic DNA was isolated from whole blood samples using genomic DNA extraction kit, following the manufacturer protocol.

Polymerase chain reaction: All PCR reaction were carried out in 15 µl reaction volume containing 100ng total genomic DNA, 0.2 µM of each primer (forward and reverse), 200 µM of dNTPs, 1X PCR buffer, 2.0 mM $MgC1_2$ and 1.0 unit of Taq DNA polymerase. Amplification conditions were initial denaturation at 95C° for 7 min, followed by 35 cycles each consisting of a denaturation of 30 sec at 94C°, annealing step of 30 sec, and an extension step of 1 min at 72C°. The last cycle was final extension at 72C° for 10 min. All amplification reactions were performed using PCR system programmable thermocycler. Each of the 31 ovine specific SSR markers (MAF65, OarFCB304, OarJMP29, OarJMP58, BM8125, OarFCB304, OarFCB128, OarCP34, OarVH72, OarHH47, DYMS1, SRCCRSP1, SRCRSP9, MCM140, MAF33, MAF209, INRA063, OarFCB20 BM1329, MAF214, ILSTS11, OarFCB226, ILSTS28, MAF70, BM1824, HUJ616, OarCP38, ILSTS5, OarAE129, SRCRSP5 and MCM527) was amplified in every individual DNA sample. Annealing temperature was adjusted accordingly for each primer. The product of PCR was analyzed on 11% polyacrylamide gel along with 50 bp DNA ladder. The gel was stained in ethidium bromide solution (0.5 µg/ml) for 20 minutes and visualized under UV light. The bands were scored manually from the gel.

Statistical analysis: Genotype for each individual was scored manually from PAGE banding pattern. Observed number of alleles, effective number of alleles, observed and expected hetrozygosity, allele frequencies (Nei 1972) and linkage disequilibrium among different combination of loci were computed using POPGENE software version 1.32 (Yeh *et al.* 1999). Allelic richness, inbreeding estimates and gene diversity at different loci were calculated using FSTAT version 2.9.3.2 (Goudet 2001). Frequencies of null alleles were calculated using software GENEPOP version 4.0 (Raymond and Rousset 1995). The allele frequency data was put in the formula given by Botstein *et al.* (1980) to calculate the polymorphism information content (PIC) for each locus. The computer software program BOTTLENECK version 1.2.02 (Piry *et al.* 1999) was used to test the mutation drift equilibrium in the population using the allele frequency data as input. The program perform 4 different tests i.e. sign rank test, standardized differences test, wilcoxon sign rank test and a qualitative test of mode shift to determine that either population is in the state of equilibrium or not.

RESULTS AND DISCUSSION

Genetic diversity within Hazaragie sheep population: Information about the amount and distribution of genetic diversity in a population can be used along with the evolutionary history of the population, to make conservation policy for endangered species (Feng 2000). Out of the total 31 SSR markers used in the study, 4 did not amplify; however, the remaining 27 markers yielded multiple bands, manifested polymorphism in Hazaragie sheep population. Different genetic parameters calculated include observed and effective number of alleles; observed, expected and average heterozygosities; gene diversity; allelic richness, polymorphism information content (PIC) and probability value for Hardy- Weinberg Equilibrium under chi-square test at each SSR marker are presented in Table 1.

Different alleles (170) was observed at 27 polymorphic loci, out of which 118.6 were effective alleles averaging 6.296 and 4.394 per locus, respectively, which is in accordance with Indian Bellary sheep (6.7 and 3.658) (Kumar *et al.* 2007), however, smaller than Italian sheep breed Gentile di Puglia (9.68) (Angelo *et al.* 2009), Indian sheep breeds Changthangi (8.76 and 4.54) (Sharma *et al.* 2010) and Munjal (8.64 and 4.57) (Yadav *et al.* 2011), and Chinese sheep breeds Ujmuqin (23.86 and 13.29), Han (20.57 and 16.45), Tan (19.86 and 11.69), Hu (18.14 and 11.77) and Tong (17.71 and 12.45) (Dongyan *et al.* 2007). The number of alleles was higher than other Indian sheep breeds i.e. Pugal (4.68 and 3.07) and Garole (4.52 and 2.92) (Mishra *et al.* 2009). Observed number of alleles (Na) ranged from 3 (OarCP34) to 11 (BM1329), while number of effective alleles (Ne) was markedly less then Na ranging from 2.571 (OarCP34) to 6.490 (BM1329). The comparison of Ne with Na at each locus provides information about the predominance of certain alleles in the population (Kumar *et al.* 2007). Value for Shannon information index (I) averaged 1.581 which is in accordance with Iranian sheep breeds Zardi (1.59), Kajal (1.57) and Kolu (1.56) (Sharifi *et al.* 2009) and lower than Iranian sheep breed Sanjabi (1.63) (Sharifi *et al.* 2009), and Indian sheep breed Munjal (1.67) (Yadav *et al.* 2011). However, value for Shannon index was higher than Indian Bellary sheep (1.419) (Kumar *et al.* 2007).

Mean observed heterozygosity (Ho) in the population was higher than Indian sheep breeds Bellary (0.512) (Kumar *et al.* 2007), Changthangi (0.691) (Sharma *et al.* 2010) and Munjal (0.712) (Yadav *et al.* 2011), ranged from 0.048 (OarCP38) to 1 (INRA63 and MAF209). Mean expected heterozygosity (He) was 0.772 and was lower than Ho at most of the loci, however, at 6 of them (OarFCB226, OarFCB20, OarCP34, BM8125, OarCP38, SRCRSP5) the value for He exceed from Ho. Similar higher heterozygosity

Table 1. Genetic diversity parameters in Hazaragie population

Locus	Sample size	Na	Ne	I	Ho	He	Hav	G_{div}	A_R	Fis	PIC	P
BM1329	45	11	6.490	2.146	0.933	0.855	0.846	0.855	4.361	–0.092	0.800	0.000
BM1824	35	6	4.246	1.538	0.800	0.776	0.765	0.775	3.519	–0.032	0.686	0.012
MAF70	37	7	4.924	1.705	0.946	0.808	0.797	0.806	3.801	–0.174	0.720	0.000
OarFCB226	37	5	4.609	1.564	0.721	0.794	0.783	0.795	3.645	0.082	0.719	0.000
OarFCB20	37	7	5.317	1.750	0.784	0.823	0.812	0.824	3.912	0.048	0.749	0.001
OarCP34	3	3	2.571	1.011	0.667	0.733	0.611	0.750	3.000	0.111	0.411	0.572
BM8125	32	4	2.764	1.156	0.531	0.648	0.638	0.650	2.825	0.183	0.451	0.001
DYMS1	36	6	5.344	1.727	0.444	0.824	0.813	0.823	3.920	–0.148	0.753	0.000
HUJ616	23	5	3.919	1.437	0.957	0.714	0.745	0.757	3.460	–0.264	0.627	0.000
ILSTS5	36	8	5.400	1.868	0.972	0.826	0.815	0.823	4.032	–0.180	0.749	0.000
ILSTS11	42	7	4.215	1.602	0.833	0.772	0.763	0.757	3.564	–0.081	0.689	0.000
ILSTS28	44	10	6.003	2.020	0.955	0.843	0.833	0.824	4.193	–0.134	0.779	0.000
INRA63	35	9	5.315	1.869	1.000	0.824	0.812	0.771	4.001	–0.218	0.750	0.000
MAF33	45	6	3.861	1.493	0.978	0.749	0.741	0.842	3.380	–0.309	0.609	0.000
MAF65	28	4	3.588	1.333	0.750	0.734	0.721	0.821	3.231	–0.022	0.651	0.000
MAF209	39	8	4.914	1.752	1.000	0.807	0.797	0.747	3.824	–0.243	0.714	0.000
MCM140	37	9	4.754	1.786	0.973	0.800	0.781	0.734	3.828	–0.219	0.747	0.000
MCM527	49	6	4.992	1.696	0.971	0.808	0.791	0.804	3.820	–0.215	0.731	0.000
OarCP38	21	5	2.981	1.291	0.048	0.682	0.666	0.798	3.092	–0.932	0.447	0.000
OarFCB193	42	5	4.552	1.563	0.786	0.781	0.780	0.806	3.633	–0.005	0.715	0.000
OarFCB304	30	6	5.405	1.736	0.900	0.829	0.815	0.698	3.956	–0.088	0.759	0.000
SRCRSP1	43	5	3.129	1.304	0.930	0.688	0.680	0.790	3.040	–0.357	–2.911	0.000
OarJMP29	36	7	3.551	1.482	0.806	0.729	0.718	0.828	3.319	–0.107	0.559	0.000
SRCRSP5	31	5	3.463	1.405	0.452	0.723	0.711	0.685	3.283	0.379	0.557	0.000
SRCRSP9	41	4	2.662	1.119	0.854	0.632	0.624	0.727	2.720	–0.357	0.522	0.000
OarJMP58	40	7	5.915	1.847	0.925	0.842	0.831	0.727	4.089	–0.101	0.776	0.000
MAF214	46	5	3.742	1.437	0.848	0.741	0.733	0.629	3.331	–0.146	0.651	0.063
Mean	36	6.296	4.394	1.581	0.825	0.772	0.757	0.772	3.584	–0.069	0.534	0.024

Key: Na, Observe number of alleles; Ne, Effective number of alleles; I, Shannon's Information index; Ho, Observed heterozygosity; He, Expected heterozygosity; Hav, Average Hetrozygosity; G_{div}, Gene diversity, A_R, Alleleic Richness; Fis, Inbreeding estimates; PIC, Polymorphism Information Content; P, probability of HW equilibrium chi square test.

is reported in Sanjabi sheep of Iran (Ho = 0.98 and He = 0.77) (Sharifi *et al.* 2009). Average heterozygosity and gene diversity were estimated to be 0.757 and 0.772, respectively, for the population. Higher values for gene diversity also reported for Italian sheep breeds Alpagota (0.806), Brogna (0.801), Foza (0.796) and Lamon (0.801) (Dalvit *et al.* 2009) are due to higher number of observed alleles in the population (Moioli *et al.* 2001). In contrast low gene diversity was reported in wild Mouflon and Bighorn sheep herds with values of 0.45 and 0.43, respectively (Saitbekova *et al.* 2001 and Forbes *et al.* 1995). Allelic richness at different loci ranged from 2.720 (SRCRSP9) to 4.361 (BM1329) averaging 3.584 for all loci. The overall inbreeding estimates (Fis) was negative (–0.069) manifesting the excess of heterozygotes in the population. Fis ranged from –0.932 (OarCP38) to 0.183 (BM8125). Lower Fis estimates are indicative of the absence of population structure. Similar results for inbreeding estimates were observed in Indian Pugal and Garole sheep populations (Mishra *et al.* 2009). In contrast, high inbreeding estimates (0.253) were calculated for Indian Bellary sheep (Kumar *et al.* 2007)

Polymorphism information content (PIC) shows the informativeness of a marker in a population genetics study. The estimated PIC values for different SSR markers in Hazaragie sheep showed that most of the markers were highly informative having value greater than 0.5. Higher PIC values are reported for Japanese sheep breeds i.e. Han 0.929, Tan 0.905 and Hu 0.902 (Sun *et al.* 2007).

When a population is able to maintain its relative allele frequencies, it is considered to be in a state of Hardy Weinberg Equilibrium (HWE). Chi-square test for Hardy Weinberg Equilibrium showed that majority of the loci and the overall population deviate significantly ($P<0.05$). This imbalanced may be attributed to deficiency in heterozygosis in the population or because of: (i) the presence of null alleles; (ii) small sample size– where rare genotypes are likely to be

Table 2. Allele's frequency at different loci in population

Locus	Alleles												
	Size	Null	A	B	C	D	E	F	G	H	I	J	K
BM1329	130–305	0.052	0.067	0.067	0.311	0.067	0.067	0.156	0.044	0.078	0.056	0.033	0.056
BM1824	170–250	0.014	0.257	0.271	0.143	0.271	0.029	0.029					
MAF70	80–250	0.059	0.027	0.027	0.257	0.230	0.243	0.095	0.122				
OarFCB226	110–150	0.131	0.243	0.162	0.216	0.270	0.108						
OarFCB20	80–140	0.035	0.027	0.162	0.243	0.221	0.149	0.162	0.027				
OarCP34	100–135	0.315	0.167	0.500	0.333								
BM8125	110–140	0.384	0.141	0.063	0.500	0.297							
DYMS1	160–235	0.047	0.083	0.236	0.222	0.208	0.111	0.139					
HUJ616	120–150	0.087	0.087	0.304	0.130	0.348	0.130						
ILSTS5	180–250	0.016	0.069	0.111	0.250	0.097	0.069	0.292	0.056	0.056			
ILSTS11	260–330	0.203	0.023	0.131	0.333	0.155	0.024	0.048	0.286				
ILSTS28	125–190	0.007	0.057	0.250	0.261	0.125	0.034	0.045	0.068	0.068	0.057	0.034	
INRA63	165–230	0.000	0.157	0.300	0.043	0.057	0.071	0.229	0.100	0.029	0.014		
MAF33	120–155	0.000	0.033	0.356	0.244	0.256	0.044	0.067					
MAF65	125–150	0.140	0.393	0.232	0.196	0.179							
MAF209	105–155	0.000	0.026	0.231	0.205	0.077	0.115	0.295	0.026	0.026			
MCM140	150–235	0.000	0.014	0.041	0.095	0.234	0.122	0.054	0.311	0.068	0.014		
MCM527	160–200	0.045	0.133	0.286	0.092	0.112	0.255	0.122					
OarCP38	340–490	0.427	0.048	0.143	0.500	0.238	0.071						
OarFCB193	90–130	0.182	0.226	0.167	0.131	0.310	0.167						
OarFCB304	160–200	0.072	0.167	0.250	0.100	0.217	0.167	0.100					
SRCRSP1	130–155	0.000	0.105	0.430	0.093	0.337	0.035						
OarJMP29	125–180	0.044	0.014	0.153	0.417	0.069	0.042	0.278	0.028				
SRCRSP5	150–200	0.223	0.129	0.097	0.436	0.258	0.081						
SRCRSP9	120–140	0.033	0.488	0.085	0.354	0.073							
OarJMP58	145–195	0.016	0.075	0.200	0.163	0.125	0.238	0.150	0.050				
MAF214	180–230	0.000	0.359	0.315	0.109	0.152	0.065						

included in the samples; (iii) the Wahlund effect– the presence of fewer heterozygotes in populations understudy than predicted; (iv) consanguinity due to inbreeding (Kumar *et al.* 2006, Kathiravan *et al.* 2009). Sample size was sufficient, however, the presence of null alleles and Wahlund effect at 6 loci may be responsible for deviating from HWE.

Test for non random association between alleles of different loci (linkage disequilibrium) showed that 25 of different allele combinations were at frequency deviating significantly from expected having $P<0.05$. However, different factors including genetic linkage, recombination rate, mutation rate, genetic drift, non-random mating, and population structure, influence the level of linkage disequilibrium (http://en.wikipedia.org/wiki/Linkage_disequilibrium). Similarly in African Merino sheep breed, 3.6% among 31, loci combinations deviated from linkage disequilibrium (Buduram 2004). Ewens-Watterson test for neutrality of the marker indicates that the reduction of heterozygosity in the population is the not the result of selection. Observed-F and values for 95% confidence intervals was calculated using 1000 simulations. The test showed that for 8 out of 27 markers the observed F value did not fall within the lower and upper level of 95% confidence interval, however, the remaining 19 markers fall within the 95% confidence interval.

Allele size and frequency: Allele frequencies at different polymorphic loci and their size range in Hazaragie population are given in Table 2. Alleles sizes for different microsatellites was within the range identified in other studies using the same loci (Yadav *et al.* 2011, Mishra *et al.* 2009 and Kumar *et al.* 2007). Null alleles were found absent at 6 loci (INRA63, MAF33, MAF209, MCM140, SRCRSP1 and MAF214) and were less frequent at 13 loci, however were at higher frequency at 8 loci with highest frequency of 0.427 at marker OarCP38. Out of total amplified 170 alleles, 59 alleles were at frequency greater than 0.2, however, 68 alleles had frequency less than 0.1 in the population. Allele B at marker OarCP34 and allele C at markers BM8125 and OarCP38 were found at highest frequency (0.5). Frequency of 4 alleles was lowest (0.014) in the population, of which 2 alleles (A and I) were at the same marker (MCM140), allele I at marker INRA63 and allele A at marker OarJMP29.

Table 3. Mutation-drift equilibrium, heterozygosity excess/deficiency under different mutation models in Hazaragie sheep population

Methods	Models	Sign test	Standardized differences	Wilcoxon sign rank test
Allele frequency method	IAM	Hee=15.86 Hd = 2 He = 25 P=0.0001	P = 0.0001	P (one tail for H deficiency) : 0.9997 P (one tail for H excess) : 0.0003 P (two tail for H excess or deficiency) : 0.0006
	TPM	Hee =15.48 Hd = 4 He = 23 P =0.0014	P=0.0000	P (one tail for H deficiency) :0.9994 P (one tail for H excess) :0.0006 P (two tail for H excess or deficiency) : 0.0012
	SMM	Hee =15.44 Hd =10 He = 17 P = 0.3397	P=0.0000	P (one tail for H deficiency) :0.8437 P (one tail for H excess) : 0.1621 P (two tail for H excess or deficiency) : 0.3242
Heterozygosity method	IAM	Hee=15.90 Hd = 1 He = 26 P = 0.0000	P= 0.0003	P (one tail for H deficiency) : 1.0000 P (one tail for H excess) :0.0000 P (two tail for H excess or deficiency) 0.0000
	TPM	Hee=15.49 Hd = 7 He = 20 P = 0.0510	P= 0.0000	P (one tail for H deficiency) : 0.9968 P (one tail for H excess) : 0.0035 P (two tail for H excess or deficiency) : 0.0070
	SMM	Hee =15.34 Hd = 14 He = 13 P = 0.2304	P= 0.0000	P (one tail for H deficiency) :0.4112 P (one tail for H excess) : 0.5980 P (two tail for H excess or deficiency) : 0.8223

Key: IAM, Infinite Allele Model; SMM, stepwise Mutation Model; TPM, two phase model; He, number of loci with heterozygosity excess; Hee, expected number of loci with heterozygosity excess; Hd, number of loci with heterozygosity deficiency; and P, probability value.

Mutation drift equilibrium: Heterozygosity excess/deficiency under different mutation models was calculated by the BOTTLENECK software, which test the mutation drift equilibrium in a population (Table 3). The results showed that there were significant number of loci with heterozygosity excess under infinite allele and 2 phase model showing lack of genetic bottleneck, however, under stepwise mutation model the value was not significant. With the reduction in the effective size of the population, the allele number is reduced faster than the heterozygosity, i.e. the observed heterozygosity is larger than the heterozygosity expected from the observed allele number when the locus is at mutation-drift equilibrium. The higher level of He with their respective Hee in both frequency and heterozygosity based methods in 2 phase and step-wise mutation models, may represent the presence of genetic bottleneck in the Hazaragie population, however, in heterozygosity based method the Hee is greater than He under step-wise mutation model.

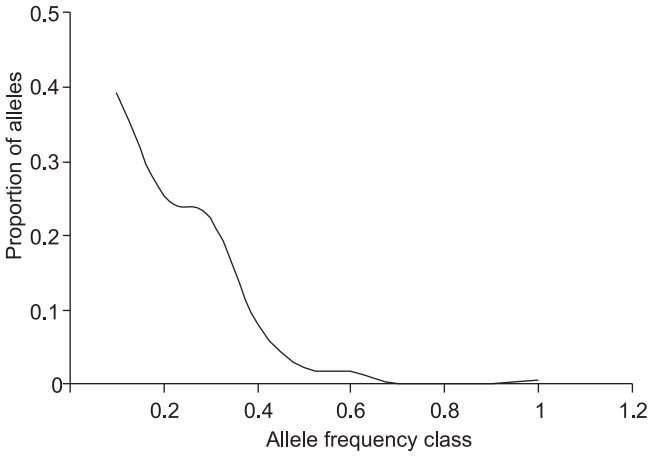

Fig. 1. Normal L-shape graph of Hazaragie population showing lack of genetic bottleneck.

In addition, qualitative graphical method of Luikart and Cornuet (1998) was also used to visualize the allele frequency spectra (Fig. 1). The microsatellite alleles were classified in to 10 frequency classes, which allow checking whether the distribution followed the normal L-shaped form, where alleles with low frequencies (0.01–0.1) are the most abundant. This reflects that the population had not undergone bottleneck at least in the recent past. Similar results were concluded for other livestock populations e.g. Marathwada buffalo

(Kathirvan *et al.* 2009) ; Muzaffarnagri, Marwari (Arora and Bhatia 2009), Michni (Ibrahim 2009), Munjal (Yadav *et al.* 2011) and Bellary sheep (Kumar *et al.* 2007) were declared free of genetic bottleneck.

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REFERENCES

Angelo F D, Albenzio M, Sevi A, Ciampolini R, Cecchi F, Ciani E and Muscio A. 2009. Genetic variability of the Gentile di Puglia sheep breed based on microsatellite polymorphism. *Journal of Animal Sciences* **87**: 1205–09.

Arora R and Bhatia S. 2009. Evaluation of genetic effects of demographic bottleneck in Muzaffarnagri sheep from India using microsatellite markers. *Asian-Australasian Journal of Animal Science* **22**:1–6.

Barker J S F, Tan S G, Moore S S, Mukherjee T K, Matheson J L and Selvaraj O S. 2001.Genetic variation within and relationships among populations of Asian goats. *Journal of Animal Breeding* **118:** 213–33.

Baumung R, Simianer H and Hoffmann I. 2004. Genetic diversity studies in farm animals – a survey. *Journal of Animal Breeding and Genetics* **121**: 361–73.

Botstein D, White R L, Sholnick M and David R. 1980. Construction of genetic linkage map in man using restriction fragment length polymorphism. *American Journal of Human Genetics* **32:** 314–31.

Buduram P. 2004. 'Genetic characterization of Southern African sheep breeds using DNA markers.' M.Sc. Thesis. Department of Animal and Wildlife, Grassland Science University Free State.

Dalvit C, De Marchi M, Zanetti E and Cassandro M. 2009. Genetic variation and population structure of Italian native sheep breeds undergoing in situ conservation. *Journal of Animal Sciences* **87**: 3837–44.

Dongyan Q U, Zhangping Y A N G, Xiaoya G U O, Yongjiang M A O, Wei S U N, Rongqing G E N, Yuehui M A, Xianglian R E N, Guobing C H A N G, Danil H U A N G and Hong C H A N G. 2007. Study on polymorphisms of microsatellite DNA of six Chinese indigenous sheep and goat breeds. *Frontier Agriculture China* **1** (4) : 472–77.

Doveri S, Lee D, Maheswaran M and Powell W. 2008. Molecular markers: History, features and applications. *Principles and Practices of Plant Genomics*. Volume 1, Pp. 23–68 (Ed.) Abbott C K A A G, Science Publishers, Enfield, USA.

FAO. 1996. Global project for maintenance of domestic animal genetic diversity (MoDAD on http://www.fao.org/dad-is/FAO.

Feng J. 2000. 'Molecular approaches for conservation of endangered giant Argali sheep *(Ovis ammon*) and dwarf blue sheep *(Pseudois nayaur schaeferi*) in Asia.' Ph.D. Dissertation, State University of New York at Buffalo.

Forbes S H, Hogg J T, Buchanan F C, Crawford A M and Allenford F W. 1995. Microsatellite evolution in cogeneric mammals: Domestic and bighorn sheep. *Molecular Biology Evolution* **12:** 1106–13.

Goudet J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). (Retrieved from: http://www.unil.ch/izea/softwares/fstat.html).

Ibrahim M. 2009. 'Morphological and molecular characterization of on-farm sheep genetic resources native to central valley of NWFP.'M.Phil Thesis, Institute of Biotechnology and Genetic Engineering, Khyber Pakhtunkhwa Agricultural University Peshawar, Pakistan.

Ibrahim M, Ahmad S, Swati Z A and Khan M S. 2010. Genetic diversity in Balkhi, Hashtnagri and Michni sheep populations using SSR markers. *African Journal of Biotechnology* **9** (45) : 7617–28.

Kathiravan P, Mishra B P, Kataria R S and Sadana D K. 2009. Eveluation of genetic architechture and mutation drift equilibrium of Marathwada buffalo population in Central India. *Livestock Science* **121:** 288–93.

Katti M V, Ranjekar P K and Gupta V S. 2001. Differential distribution of simple sequence repeats in eukaryotic genome sequences. *Molecular Biology Evolution* **18**: 1161–67.

Kumar D, Sharma R, Pandey A K, Gour D S, Malik G, Ahlawat S P S and Jain A. 2007. Genetic diversity and bottleneck analysis of Indian Bellary sheep by microsatellite markers. *Russian Journal of Genetics* **43:** 996–1005.

Kumar S, Gupta T, Kumar N, Dikshit K, Navani N, Jain P and Nagarajan M. 2006. Genetic variation and relationships among eight Indian riverine buffalo breeds. *Molecular Ecology* **15:** 593–600.

Luikart G and Cornuet J M. 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conservation Biology* **12** (1) : 228–37.

Mishra B, Tantia M S and Vijh R K. 2009. Genetic differentiation and structuring of Pugal and Garole sheep. *Indian Journal of Animal Sciences* **79** (4) : 392–98.

Mishra P, Verma N K, Aggarwal R A K and Dixit S P. 2010. Breed characteristics and genetic variability in Changthangi goats. *Indian Journal of Animal Sciences* **80** (12) : 1203–09.

Moioli B, Georgoudis A, Napolitano F, Catillo G, Giubilei E, Ligda C H and Hassnane M. 2001. Genetic diversity between Italian, Greek and Egyptian buffalo populations. Livestock Production Science **70:** 203–11.

Nei M. 1972. Genetic distance between populations. *American Naturalist*. **106:** 280–92.

Peter R and Blood E D. 2001. *Afghanistan: A Country Study.* Washington: GPO for the Library of Congress.

Piry S, Luikart G and Cornuet J M. 1999. Bottleneck: a computer program for detecting recent reduction in the effective population size using allele frequency data. *Journal of Heredity* **90:** 502–03.

Rashiq M H. 2004. *Afghanistan: sheep and Goat Management*. Kabul University. Pp.48–59.

Raymonds M and Rousset F. 1995. GENEPOP (version 4.0) : population genetic software for exact test and ecumenicism. *Journal of Heredity* **86:** 248–49.

Saitbekova- stahlberger N, Schlapfer j, Dolf G and Gaillord C. 2001. Genetic relationshps in Swiss sheep breeds based on microsatellite analysis. *Journal of Animal Breeding and Genetics* **118:** 379– 87.

Scherf B D. 2000. *World Watch List for Domestic Animal Diversity*. 3rd edn.

Sharifi Seidani E, Amirinia C, Lavaf A, Farasati C and Aminafshar

M. 2009. Genetic variation among different ecotypes of the Iranian Sanjabi sheep. *Journal of Animal and Veterinary Advancement* **8:** 1173–76.

Sharma R, Pandey A K, Singh L V, Maitra A, Arora R, Bhatia S and Mishra B P. 2010. Microsatellite based diversity estimation of Changthangi – a high altitude sheep breed of India. *Indian Journal of Animal Sciences* **80** (5) : 436–40.

Soysal M I, Tuna Y T, Ozkan E, Gurchan E K, Togan I and Altunok V. 2005. Study on the wool characteristics of several Turkish sheep breed according to microsatellite DNA type. *Pakistan Journal of Biological Sciences* **8:** 186–89.

Tautz D and Schlotterer C. 1994. Concerted evolution, molecular drive and natural-selection reply. *Current Biology* **4:** 1166–66.

Toth G, Gaspari Z and Jurka J. 2000. Microsatellites in different eukaryotic genomes: Survey and analysis. *Genome Research* **10:** 967–81.

Sun W, Chang H, Ji D, Liao X, Du L, Lu S and Kenji T. 2007. Analysis on genetic diversity and isolation mechanism by distance of different ecological type sheep breeds in Mongolia sheep group. *Journal of Genetics and Genomics* **34** (11) : 1001–09.

Vignal A, Milan D, SanCristobal M and Eggen A. 2002. A review on SNP and other types of molecular markers and their use in animal genetics. *Genetic Selection and Evolution* **34**: 275–305.

Vijh R K, Tantia M S, Behl R and Mishra B. 2010. Genetic architecture of Black Bengal and Chegu goats. *Indian Journal of Animal Sciences* **80** (11) : 1134–37.

Yadav D K, Arora R, Bhatia S and Singh G. 2011. Short tandem repeat based analysis of genetic variability in Munjal – the threatened sheep population of Northwestern India. *Indian Journal of Animal Sciences* **81** (2) : 171–75.

Yeh F C, Yang R C and Boyle T. 1999. Microsoft windows based freeware for population genetic analysis. *POPGENE version 1.3.1*, Molecular biology and technology center, University of Albeta, Canada.