



Genetic diversity analysis of native sheep population of Eastern Indian Himalayas using microsatellite DNA markers

ARUNDHATI PHOOKAN^{1✉}, GALIB UZ ZAMAN¹, BULA DAS¹, ARPANA DAS¹, PRANAB JYOTI DAS²,
MOMI SHARMA¹, SHEIKH FIRDOUS AHMAD³ and SOURABH DEORI⁴

Assam Agricultural University, Khanapara, Guwahati, Assam 785 013 India

Received: 17 July 2023; Accepted: 19 April 2024

ABSTRACT

A study was undertaken for genetic diversity analysis of three native sheep populations of Eastern Indian Himalaya utilizing twenty five ovine microsatellite markers. A total of 150 sheep, 50 each from the states of Assam, Meghalaya and Arunachal Pradesh were genotyped through microsatellite. Polymorphism was revealed in most of the markers with a total of 242 alleles. The average observed (H_o) and expected (H_e) heterozygosity estimates for Assam, Meghalaya and Arunachal Pradesh sheep were 0.311 and 0.396; 0.323 and 0.383; and 0.416 and 0.408, respectively suggesting that the genetic diversity of each population ranged from intermediate to high. The average polymorphism information content (PIC) estimates and Shannon's Information index were 0.357 and 0.70, 0.366 and 0.75, and 0.292 and 0.75 in Assam, Meghalaya and Arunachal Pradesh sheep populations. Mean F_{IS} (within-population inbreeding estimate), F_{IT} (Between-population inbreeding estimate) and F_{ST} (Population differentiation measure) estimate for three populations under consideration were 0.149, 0.224 and 0.088, respectively. Significant deviations from Hardy-Weinberg equilibrium (based on chi-square) were found in a total of 10, 13 and 6 alleles in Assam, Meghalaya and Arunachal Pradesh sheep population which indicated evolutionary force in operation at these loci in the respective population. The study revealed that Assam sheep and Meghalaya sheep are the closest relatives whereas Assam sheep and Arunachal Pradesh sheep are farthest relatives.

Keywords: Genetic diversity analysis, Indigenous sheep microsatellite markers, Phylogenetic tree.

Sheep is an important species of livestock in India that contribute greatly to the agrarian economy. They play an important role in the livelihood of a large percentage of small, marginal farmers and landless labourers. Furthermore, sheep farming is a logical source of livelihood when crop production is uncertain or crop failure occurs. In the Eastern Indian Himalaya region, sheep are mostly reared for meat purposes and are devoid of any religious prejudice on mutton consumption. Assam, Meghalaya and Arunachal Pradesh are the states in Eastern Indian Himalaya having the highest sheep population as compared to other states. The total indigenous sheep population in Assam, Meghalaya and Arunachal Pradesh are 332100, 15580 and 7085 (Twentieth Livestock census) respectively. In Assam, out of the total sheep population, more than 90% are indigenous sheep. They are distributed in Dhubri, Barpeta, Darrang, Kamrup, Bongaigaon and Goalpara districts of

Assam. *Asomi Bhera*, the indigenous sheep of Assam is a small-sized animal predominantly of light brown body coat colour with an average adult body weight of 13.457 ± 0.27 kg having high reproductive efficiency and fecundity with mean lamb size of 2.3 and 67.98 per cent twins (Nath 2018). Indigenous sheep of Meghalaya are distributed mostly in East Khasi Hills, West Khasi Hills, West Garo Hills and South West Garo Hills district. They are medium-sized animals with average adult body weight of 20.21 ± 0.05 kg and mostly light brown in body coat colour, having good potential for mutton production and possess coarse wool type which can be utilized in the carpet weaving industry (Khanikar 2020). Indigenous sheep of Arunachal Pradesh are mainly found in Tawang and West Kameng district of Arunachal Pradesh which are at an altitude of 7,802 ft and 8,757 from the sea level, respectively. They are medium-sized mostly white with black or brown face with an adult mean body weight of 21 kg and produce excellent lustrous carpet quality wool. It is very unfortunate that genetic diversity of these indigenous sheep is not well known. Determination of status and characterization of indigenous sheep genetic resources is essential for planning domestic animal diversity conservation. Microsatellites are the most popular markers recommended by FAO for livestock genetic characterization studies (FAO 2012). Indeed,

Present address: ¹College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam. ²ICAR–National Research Centre on Pig, Rani, Guwahati, Assam. ³ICAR–Indian Veterinary Research Institute, Bareilly, Uttar Pradesh. ⁴ICAR–Research Complex for North Eastern Hill Region, Umiam, Meghalaya. ✉Corresponding author email: arundhatiphookan@gmail.com

microsatellites are deemed to be one of the most valuable genetic markers for genetic characterization

Hence, the present study planned for molecular characterization of indigenous sheep of the Eastern Indian Himalayan region and to elucidate the degree and pattern of genetic

MATERIALS AND METHODS

Sampling and genomic DNA extraction: Three states in the eastern Indian Himalayan region, viz. Assam, Meghalaya and Arunachal Pradesh were selected based on the population density of indigenous sheep. A total of 150 blood samples, 50 samples from each state were collected randomly from apparently healthy and unrelated indigenous sheep. Genomic DNA was isolated using phenol chloroform extraction procedure (Sambrook and Russell 2001) with slight modifications using DNAzol reagent instead of SDS and proteinase K.

Genotyping by microsatellite markers: A battery of twenty-five microsatellite markers was selected from FAO-ISAC recommended list (Table 1). The forward primer for each marker was fluorescently labelled with FAM, PET, VIC and NED dye. The PCR conditions were optimized for all of the 25 microsatellite primers for the amplification of isolated genomic DNA. The amplification was carried out in Thermal Cycler (Applied Biosystem, USA). Polymerase Chain Reaction (PCR) was carried out on about 100 ng genomic DNA in a 30 µl reaction volume. The reaction

mixture consisted of 10 µl nuclease-free water, 26 µl master mix (Thermo fisher Scientific), 1 µl forward primer (10 pmol/µl), 1 µl reverse primer (10 pmol/µl) (Thermo fisher Scientific) and 2 µl DNA Template (100 ng). The cycling protocol used was initial denaturation of 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 sec, annealing at different temperatures (depending on the primer) for 45 s, extension at 72°C for 50 s, final extension at 72°C for 10 min and hold at 4°C for ∞. The PCR products were checked for amplification by loading on 2% agarose gel. A 50 bp ladder was loaded alongside a molecular size marker and checked for amplification on Gel Documentation System (Molecular Imager, Gel DOC™ XR⁺, BIO-RAD).

Amplicons were sized by fragment analysis on ABI automated DNA sequencer and typing of the individual sheep at 25 microsatellite loci was carried out. The post PCR multiplexing was used to simultaneously genotype 3 or 4 loci depending upon the size and dye label of the PCR product. The sizing and allele calling was performed using Genotyper ver. 3.0 software (Applied Biosystems).

Statistical analysis: The derived microsatellite information was utilised for statistical analysis by using POPGENE 32 software (Yeh *et al.* 1995). Intra-population genetic variation of the microsatellites was quantified using the allele frequencies, observed and effective number of alleles (Nei 1987). The heterozygosity measures were calculated using the formulae given by Levene (1949).

Table 1. List of microsatellite primers selected for the genetic diversity analysis in sheep population

Locus	Dye	Annealing Temp. (°C)	Allele size	Reference details for allele size
OarHH47	VIC	59	130-152	FAO 2011
OarVH72	FAM	64	121-145	FAO 2011
OarAE129	NED	61	133-159	FAO 2011
MAF214	PET	65	174-282	FAO 2011
OarFCB304	NED	63	150-188	FAO 2011
OarFCB48	PET	60	144 160	Pramod <i>et al.</i> 2009
OarJMP29	FAM	60	124-148	Pramod <i>et al.</i> 2009
BM6526	VIC	62	154-172	Pramod <i>et al.</i> 2009
OarFCB128	PET	60	96-130	FAO 2011
OarHH64	PET	62	118-136	Pramod <i>et al.</i> 2009
OarJMP58	HEX	62	130-168	Ramachandra <i>et al.</i> 2015
OarCP20	PET	61	74-84	Pramod <i>et al.</i> 2009
SRCRSP9	FAM	54	112-140	FAO 2011
OarFCB20	HEX	56	95-120	FAO 2011
CSSM47	VIC	54	132-162	Pramod <i>et al.</i> 2009
OarJMP8	FAM	58	110-134	Pramod <i>et al.</i> 2009
BM757	FAM	56	172-196	Pramod <i>et al.</i> 2009
SRCRSP5	FAM	54	126-158	FAO 2011
MAF70	HEX	65	124-166	FAO 2011
OarCP34	FAM	58	110-128	FAO 2011
HUJ616	VIC	52	114-160	FAO 2011
OarHH35	NED	58	118-138	Pramod <i>et al.</i> 2009
BM8125	FAM	59	107-131	FAO 2011
OarCP38	FAM	59	112-130	FAO 2011
INRA63	FAM	58	167-203	Sharma <i>et al.</i> 2010

The F statistics values F_{IS} , F_{IT} and F_{ST} were estimated (Nei 1987). Nei's unbiased measures of genetic identity and genetic distances were estimated following the method of Nei (1978).

RESULTS AND DISCUSSION

Allele diversity: Allele size range, number of observed alleles and effective number of alleles in three populations of indigenous sheep from eastern Indian Himalaya are presented in Table 2. The total number of alleles scored in the present study at different polymorphic microsatellite loci in three sheep populations was 242, with contributions of 79, 84 and 79 alleles from Assam, Meghalaya and Arunachal Pradesh sheep population, respectively. Six loci each in Assam and Meghalaya sheep and eight loci in Arunachal Pradesh sheep were found to be monomorphic for which no subsequent analysis was done. Similar reports of 242 alleles scored in different sheep breed was reported by Amareswari *et al.* (2018) in Deccani (254 alleles) and Nellore sheep breed (260 alleles) and Molabanti *et al.* (2019) in Macherla Brown sheep breed (275 alleles). However, lower total alleles scored by Ravimurugan (2015) in Madras Red sheep breed (175 alleles) and Martínez *et al.*

(2021) in Mexican Pelibuey sheep population (99 alleles); and a higher total allele was scored in Deccan sheep (407 alleles) by Yadav *et al.* (2017).

In the present study, the number of alleles varied from one to eight. The most polymorphic locus in Assam population was MAF70 (7alleles).Whereas, MAF70 and OarHH47 are the most polymorphic loci in sheep of Meghalaya and Arunachal Pradesh population with 8 alleles each. A relatively similar finding for number of alleles was reported by Arora *et al.* (2008) and Pramod *et al.* (2009) in Jalauni and Vembur sheep breeds which ranged from two to nine. However, a higher range was reported by Amareswari *et al.* (2018) as four to fourteen in Deccani and Nellore sheep breed; Molabanti *et al.* (2019) as eight to eighteen in Macherla Brown sheep breed and Martínez *et al.* (2021) as nine to fourteen in Mexican Pelibuey sheep population

The observed and effective numbers of alleles are also an index used to reveal the genetic diversity of the populations. The mean observed numbers of alleles (N_a) were found to be 3.160, 3.360 and 3.160 in Assam, Meghalaya and Arunachal Pradesh sheep respectively. The mean N_a in all the three sheep, populations over 25 loci

Table 2. Allele size range, number of observed alleles and effective number of alleles in three populations of indigenous sheep from Eastern Indian Himalaya

Locus	Allele size range (bp)	Assam		Meghalaya		Arunachal Pradesh		Mean	
		N_a	N_e	N_a	N_e	N_a	N_e	N_a	N_e
OarHH47	126-146	6	2.93	8	4.45	8	5.08	7.33	4.15
OarVH72	117-140	3	2.37	6	2.13	7	3.75	5.33	2.75
OarAE129	140-154	6	3.87	7	3.21	5	2.62	6	3.23
MAF214	184-199	3	2.19	3	2.02	2	1.88	2.67	2.02
OarFCB304	136-184	5	3.10	5	3.75	4	3.73	4.67	3.52
OarFCB48	142-166	1	1	1	1	1	1	1	1
OarJMP29	132-145	1	1	1	1	1	1	1	1
BM6526	154-172	1	1	1	1	1	1	1	1
OarFCB128	96-134	3	1.17	3	2.45	5	3.43	3.67	2.35
OarHH64	121-132	4	2.19	2	1.60	3	1.74	3	1.84
OarJMP58	145-165	4	1.10	4	2.50	4	2.29	4	2.26
OarCP20	69-91	1	1	1	1	1	1	1	1
SRCRSP9	110-120	4	1.30	3	1.59	1	1	2.67	1.30
OarFCB20	110-120	3	2.37	4	1.74	4	2.82	3.67	2.31
CSSM47	132-160	3	1.98	3	1.31	1	1	2.33	1.43
OarJMP8	115-130	4	2.35	4	2.90	3	1.91	3.67	2.39
SRCRSP5	148-156	2	1.06	2	1.49	3	1.46	2.33	1.34
MAF70	130-160	7	4.52	8	4.83	8	5.57	7.67	4.97
OarCP34	110-128	5	3.24	4	2.37	3	2.28	4	2.63
HUJ616	114-160	2	1.75	2	1.49	2	1.34	2	1.53
OarHH35	117-137	4	3.15	5	3.52	5	4.63	4.67	3.77
BM757	178-198	3	1.51	3	2.14	3	2.72	3	2.12
BM8125	107-131	2	2	2	2	2	2	2	2
OarCP38	112-130	1	1	1	1	1	1	1	1
INRA63	167-203	1	1	1	1	1	1	1	1
Mean	-	3.16	2.04	3.36	2.14	3.16	2.29	3.23	2.16
SD	-	1.75	0.99	2.16	1.10	2.19	1.38	1.93	1.10

N_a , Observed number of alleles; N_e , Expected number of alleles.

Table 3. Population wise observed and expected heterozygosity for the 25 microsatellite loci

Locus	Assam		Meghalaya		Arunachal Pradesh		Mean	
	H _o	H _e	H _o	H _e	H _o	H _e	H _o	H _e
OarHH47	0.53	0.67	0.73	0.79	0.81	0.82	0.74	0.76
OarVH72	0.09	0.59	0.18	0.53	0.70	0.75	0.32	0.62
OarAE129	0.56	0.75	0.68	0.69	0.63	0.63	0.62	0.69
MAF214	0.16	0.55	0.23	0.51	0.07	0.47	0.15	0.51
OarFCB304	0.50	0.69	0.53	0.74	0.67	0.74	0.56	0.72
OarFCB48	*	*	*	*	*	*	*	*
OarJMP29	*	*	*	*	*	*	*	*
BM6526	*	*	*	*	*	*	*	*
OarFCB128	0.03	0.15	0.03	0.60	0.20	0.72	0.09	0.49
OarHH64	0.44	0.55	0.15	0.38	0.30	0.43	0.29	0.45
OarJMP58	0.69	0.51	0.68	0.61	0.67	0.57	0.68	0.56
OarCP20	*	*	*	*	*	*	*	*
SRCRSP9	*	0.23	*	0.38	*	*	*	0.20
OarFCB20	0.60	0.59	0.27	0.43	0.87	0.66	0.57	0.56
CSSM47	0.44	0.50	0.26	0.24	*	*	0.23	0.25
OarJMP8	0.09	0.58	0.68	0.66	0.37	0.48	0.38	0.58
SRCRSP5	0.06	0.06	0.06	0.33	0.30	0.32	0.14	0.24
MAF70	0.72	0.79	0.79	0.80	0.87	0.83	0.79	0.81
OarCP34	0.60	0.70	0.41	0.59	0.60	0.57	0.53	0.62
HUJ616	0.37	0.44	0.18	0.33	0.23	0.26	0.26	0.34
OarHH35	0.66	0.69	0.73	0.73	0.70	0.80	0.70	0.74
BM757	0.25	0.34	0.50	0.54	0.60	0.64	0.45	0.51
BM8125	1	0.51	1	0.51	1	0.51	1	0.51
OarCP38	*	*	*	*	*	*	*	*
INRA63	*	*	*	*	*	*	*	*
Mean	0.32	0.40	0.32	0.42	0.38	0.41	0.34	0.41
St. Dev	0.06	0.06	0.06	0.06	0.07	0.06	0.06	0.06

H_o, Observed heterozygosity; H_e, Expected heterozygosity; *, Monomorphic marker loci.

was found to be 3.227. The mean effective allele number (N_e) or overall expected number of alleles was found to be 2.042, 2.139 and 2.290, respectively for Assam, Meghalaya and Arunachal Pradesh sheep. The mean N_e in all the three sheep populations over 25 loci was found to be 2.157. N_a and N_e revealed intermediate genetic polymorphism in the investigated sheep populations. A higher value was reported by Amareswari *et al.* (2018), Sharma *et al.* (2020) and Molabanti *et al.* (2021) in different Indian breeds of sheep.

Genetic variability: Heterozygosity is one of the indices used to assay the genetic variation of each population. The values of heterozygosity indicate the diversity level of the molecular markers. When the value is high, the molecular marker's diversity is high too. To describe the genetic diversity within the populations, the observed (H_o) and expected heterozygosities (H_e) were calculated. The summary statistics of heterozygosity for 25 microsatellite loci is presented in Table 3. The mean H_o was found to be 0.317, 0.323 and 0.383 in Assam, Meghalaya and Arunachal Pradesh sheep. The corresponding mean H_e values were found to be 0.396, 0.417 and 0.408, respectively. The overall average H_o and H_e of all populations for all the loci were 0.341 and 0.407, respectively. The mean H_o was lower

than the mean H_e in all the three populations studied. Among all the loci studied, the H_o and H_e of the locus MAF 70 had the highest value (0.793 and 0.810). The genetic diversity of each population ranged from intermediate to high. This may be related to the breeding history and environment of each population. The districts of Arunachal Pradesh where the indigenous sheep are available are very far from Assam and Meghalaya. Also, the sheep from the states under study are thriving in different environment in terms of altitude and temperature. A higher value compared to the present findings were reported by Singh *et al.* (2017), Amareswari *et al.* (2018) and Sharma *et al.* (2020) in different sheep breeds of India.

Polymorphism information content (PIC): The PIC was a good index for genetic diversity evaluation. The PIC was calculated to measure the genetic diversity at each of the polymorphic loci and presented in Table 4. The mean PIC value for Assam sheep population was found to be 0.357 and the PIC value was the highest for MAF70 (0.745) and lowest for SRCRP5 (0.059). In Meghalaya sheep, the average PIC value was found to be 0.366 with the highest PIC value for MAF70 locus (0.769) and the lowest value for CSSM47 locus (0.223). In Arunachal Pradesh sheep, the average PIC value was found to

Table 4. Mean PIC, Shanon Index and global F-statistics and number of migrants across different sheep populations

Locus	Mean PIC	Mean Shanon Index	F_{IS}	F_{IT}	F_{ST}	N_m
OarHH47	0.708	1.621	0.074	0.145	0.077	3.007
OarVH72	0.554	1.165	-0.238	-0.143	0.076	3.018
OarAE129	0.638	1.373	0.624	0.642	0.047	5.035
MAF214	0.395	0.752	-0.169	-0.152	0.015	16.441
OarFCB304	0.664	1.351	0.474	0.503	0.056	4.215
OarFCB48	*	*	0.086	0.147	0.067	3.492
OarJMP29	*	*	0.697	0.705	0.027	9.075
BM6526	*	*	0.208	0.237	0.037	6.536
OarFCB128	0.443	0.904	0.235	0.322	0.114	1.938
OarHH64	0.377	0.734	0.342	0.400	0.088	2.599
OarJMP58	0.479	0.962	-0.222	-0.150	0.059	4.007
OarCP20	*	*	0.820	0.861	0.231	0.831
SRCRSP9	0.180	0.380	1	1	0.085	2.702
OarFCB20	0.488	0.967	-0.047	0.137	0.175	1.175
CSSM47	0.203	0.406	0.041	0.232	0.199	1.007
OarJMP8	0.496	0.983	0.333	0.473	0.210	0.940
SRCRSP5	0.208	0.415	0.400	0.436	0.059	3.977
MAF70	0.771	1.769	0.005	0.083	0.078	2.968
OarCP34	0.542	1.088	0.123	0.245	0.139	1.553
HUJ616	0.278	0.517	0.224	0.245	0.026	9.277
OarHH35	0.677	1.375	0.042	0.064	0.024	10.286
BM757	0.445	0.856	0.102	0.174	0.080	2.863
BM8125	0.375	0.693	-1	-1	0	0
OarCP38	*	*	0	0	0	0
INRA63	*	*	0	0	0	0
Mean	0.357	0.732	0.166	0.224	0.079	3.877
St. Dev	0.255	0.548	0.398	0.394	0.066	3.861

F_{IS} , Within-population inbreeding estimate; F_{IT} , Between-population inbreeding estimate; F_{ST} , Population differentiation measure; N_m , Number of migrants; *, Monomorphic marker loci.

be 0.362 with the highest PIC value for MAF70 locus (0.798) and the lowest value for HUJ616 locus (0.222). Among all the microsatellite loci, the locus MAF70 had the highest PIC (0.771), while the locus SRCRSP9 had the lowest PIC (0.180). The average PIC value of all sites considering all the three populations was found to be 0.357. The average PIC of the 25 microsatellite sites for all three sheep populations ranged between 0.180 and 0.771. This indicated that the selected microsatellite loci had intermediate to high diversity in all the sheep populations and can reflect the genetic relationship of these different populations on a molecular level. The polymorphic nature of the microsatellite markers made them markers of choice in studying the characterization and genetic diversity of the three sheep populations. However, Singh *et al.* (2017) and Amareswari *et al.* (2018) observed a higher PIC value compared to the present finding.

Shannon's information index: Shannon's information index is presented in Table 4. The results showed a high level of Shannon's information index with an average value of 0.698, 0.749 and 0.750 in Assam, Meghalaya and Arunachal Pradesh sheep, respectively. Shannon's information index showed that most of the loci were highly informative indicating high polymorphism of all

loci with an overall average of 0.732 when all the loci and populations were considered together. A higher value was given by Ravimurugan (2017) and Sharma *et al.* (2020) in sheep breeds of India.

Fixation index (F_{IS} , F_{ST} , and F_{IT}): F_{IS} , F_{ST} and F_{IT} estimates are considered analogous to global inbreeding estimate (f), total inbreeding estimate (F) and population differentiation estimate, respectively. The F-statistics are depicted in Table 4. The F_{IS} and F_{IT} may be positive or negative values, however, the F_{ST} values are always positive. When there is no differentiation, the value of F_{ST} is 0; when alleles among populations are quite different, the value of F_{ST} equals 1.

F_{IS} : F_{IS} shows the proportion of the variance in the subpopulation contained in an individual. More F_{IS} means more variation so more heterozygosity. The mean F_{IS} estimate for three breeds was very low (0.166) implying an overall significant heterozygote deficit. The within breed deficit in heterozygosity, as evaluated by the F_{IS} parameter, ranged from -1 (BM812) to 0.820 (CP20). The observed F_{IS} estimate was following those reported in other domestic sheep breeds investigated earlier (Molaei *et al.* 2009, Radha *et al.* 2011). However, lower value F_{IS} was observed by Amareswari *et al.* (2018) and Sharma *et al.* (2020) in different sheep breeds and a higher F_{IS} was reported by

Table 5. Nei's genetic identity and distance measures among different sheep populations under study

Population	Assam	Meghalaya	Arunachal Pradesh
Assam	-	0.905	0.803
Meghalaya	0.099	-	0.874
Arunachal Pradesh	0.219	0.135	-

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Pramod *et al.* (2009) in Vembur sheep.

F_{ST} : The F_{ST} determines the genetic differentiation among the populations, corresponding to nearly 0.079 of the genetic variability when all the loci were considered. The F_{ST} value ranged between 0.015 (MAF 214) and 0.210 (OarJMP8) for each locus individually. The average F_{ST} estimate pointed towards the least differentiation of the population under study. Observations on F_{ST} reported by Ramachandran *et al.* (2015) and Sharma *et al.* (2020) was in accordance with the present finding. However, Dashab *et al.* (2011) observed a lower F_{ST} value in Baluchi sheep.

F_{IT} : The divergence between H_e and H_o for all the individuals, as reflected by F_{IT} had an overall average of 0.224 for all the loci studied. The range for different markers was between -1 (BM8125) and 0.861 (OarCP20). F_{IT} observed in the present report was found to corroborate with the finding of Sharma *et al.* (2020). However, Ramachandran *et al.* (2015) observed a lower overall mean F_{ST} in sheep breeds, viz Madras Red, Mecheri, Pattanam and Nellore and Dashab *et al.* (2011) in Baluchi sheep.

N_m : The mean number of migrants in the three populations under consideration was found to be 3.877 (Table 4). However, a lower value was observed by Sharma *et al.* (2020) in a study on 11 sheep breeds of India as 2.000 ± 0.160 .

Hardy-Weinberg Equilibrium (HWE): The test of HWE was used to judge whether the genotypes were maintained in balance or deviated from balance. A total of 19 microsatellite marker loci were found to be polymorphic in Assam and Meghalaya sheep among 25 markers studied. In Arunachal Pradesh sheep a total of 17 microsatellite marker loci were found to be polymorphic. All polymorphic loci were tested for deviations from Hardy-Weinberg equilibrium using chi-square. Significant deviations from Hardy-Weinberg equilibrium were found in a total of 10, 13 and 6 alleles in Assam, Meghalaya and Arunachal Pradesh sheep population, respectively. Significant deviations from HWE were indicative of evolutionary force in operation at these loci in the respective population. Selection, non-random mating and inbreeding were the main reasons which induced the disequilibrium. Moreover, other reasons were causing the disequilibrium, such as mutation, genetic drift, operation of selection over years, small population size, less number of male individuals, etc. The status of sheep population in Meghalaya and Arunachal Pradesh was small which may lead to genetic drift as well as inbreeding. In the sheep population of Assam, selection

has been done for many years without introduction of any other breed. Further, as sheep are let loose for grazing and only few males are available hence same male animal mated with females of many households. Arora *et al.* 2008 also observed significant deviation ($P < 0.05$) from HWE at six loci in the Jalauni sheep breed which is following the finding observed in Arunachal Pradesh sheep. Further, Pramod *et al.* (2009) observed that 19 out of 25 loci deviated from Hardy-Weinberg equilibrium in Vembur sheep and Ravimurugan (2015) observed 75% of loci showed deviation from HWE in Madras Red sheep.

Genetic distances and clustering of sheep: Nei's genetic identity and genetic distances among populations are presented in Table 5. Genetic identity was found to be 0.905, 0.874 and 0.803 between Assam and Meghalaya sheep; Meghalaya and Arunachal Pradesh sheep and Assam and Arunachal Pradesh sheep, respectively. The genetic distance between Assam and Arunachal Pradesh sheep (0.219) was longest followed by Meghalaya and Arunachal Pradesh sheep (0.135) and Assam and Meghalaya sheep (0.099), respectively. Nei's genetic distance (Nei 1978) was utilized for the construction of a phylogenetic tree by UPGMA for finding out the relationship among three sheep populations as shown in Fig. 1. The tree revealed that Assam and Meghalaya sheep falls in same clade and Arunachal Pradesh sheep was found to be totally different and unique. The phylogenetic tree showed that the branch length of Arunachal sheep from root was 8.88 and the branch length of Assam sheep and Meghalaya sheep from the node was 5.01.

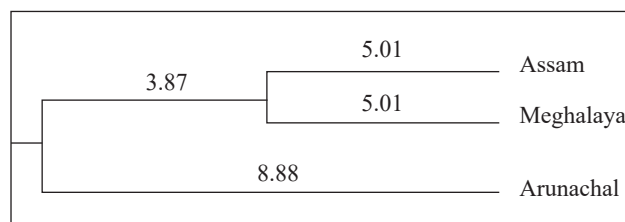


Fig. 1. UPGMA base dendrogram showing phylogenetic relationship between three populations under study.

Molecular characterization and genetic diversity study of indigenous sheep of Eastern Indian Himalaya using microsatellite DNA markers revealed a moderate/intermediate genetic diversity in the sheep population under study which may be due to unplanned mating as the sheep are let loose for grazing by the farmers and animals are mated in the field condition without any selection, small population or closed herd. Assam and Meghalaya sheep are the closest relatives followed by Assam and Meghalaya sheep to Arunachal sheep. Lack of differences between sheep population especially between Assam and Meghalaya may be due to the fact that these two states are adjacent to each other and share common boundary bearing close geographical distance and similar ecology which may had lead to migration of animals from one population to other and thus gene flow between them leading to

uncontrolled breeding. However, the sheep belonging to state of Arunachal Pradesh are unique population.

REFERENCES

- Amareswari P, Gnana Prakash M, Ekambaram B, Mahendar M and Hari Krishna Ch. 2018. Molecular genetic studies on Nellore and Deccani sheep using microsatellite markers. *Indian Journal of Animal Research* **52**: 805–10.
- Arora R and Bhatia S. 2006. Genetic Diversity of Magra sheep from India using microsatellite analysis. *Asian-Australian Journal of Animal Science* **19**: 938–42.
- Dashaba G R, Aslaminejada A, Nassiria M, Esmailizadeh A K and Saghia D A. 2011. Analysis of genetic diversity and structure of Baluchi sheep by microsatellite markers. *Tropical Subtropical Agroecosystems* **14**: 1047–054
- FAO. 2011. Guidelines on molecular genetic characterization of animal genetic resources. Available online at: <http://www.fao.org/docrep/>.
- Hall TA. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Ser* **41**: 95–98.
- Khanikar and Dimpi (2020). 'Performance evaluation and polymorphism profiling of fecundity genes in indigenous sheep of Meghalaya.' MVSc thesis submitted to the Assam Agricultural University.
- Levene H. 1949. On a matching problem arising in genetics. *Annals of Mathematical Statistics* **20**: 91–94.
- Martínez C U A, Gutiérrez B E, Correa J C J, Villalobos J M B, Méndez J V and Roldán A R, 2021. Genetic characterization of Mexican Pelibuey sheep using microsatellite markers. *La Revista Mexicana de Ciencias Pecuarias* **12**: 36–57.
- Molabanti M, Bhupati P, Jeepalyam S, Gutti B and Talokar A J, 2019. Genetic diversity analysis of macherla brown sheep using microsatellite markers. *International Journal of Livestock Research* **9**: 84–93.
- Molaeae V, Osfoori R, EskandariNasaba M P and Qanbari S. 2009. Genetic relationships among six Iranian indigenous sheep populations based on microsatellite analysis. *Small Ruminant Research* **84**:121–24.
- Musthafa M M, Aljummah R S and Alshaik M A. 2012. Genetic diversity of Najdi sheep based on microsatellite analysis. *African Journal Biotechnology* **11**:14868-4876.
- Nath Susmita. 2018. 'Characterization of indigenous sheep (*Ovis aries*) of Assam.' MVSc thesis submitted to the Assam Agricultural University.
- Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**: 583–90.
- Nei M. 1987. Molecular Evolutionary Genetics. Columbia University Press. New York.
- Pramod S, Kumarasamy P, Rosalyn Mary Chandra A, Sridevi P and Rahumathulla P S. 2009. Molecular characterization of Vembur sheep (*Ovis aries*) of south India based on microsatellites. *Indian Journal of Science and Technology* **2**: 55–08.
- Radha P, Sevasalvam S N, Kumaraswamy P and Kumanan K. 2011. Genetic diversity and bottle neck analysis of Kilakarsal sheep by microsatellite markers. *Indian Journal of Biotechnology*. **10**: 52–53.
- Ramachandran A, Thiruvankadan A K, Kathiravan P, Saravanan R, Panneerselvam S and Elango A. 2015. Microsatellite-based phylogeny of Indian sheep breeds. *Indian Journal of Animal Sciences* **85**:1209–214.
- Ravimurugan T. 2015. Molecular characterization of Madras Red sheep using microsatellite markers. *Indian Journal of Small Ruminant* **21**: 200–03
- Ravimurugan T. 2017. Genetic diversity analysis of Kilakarsal sheep by microsatellite markers. *International Journal of Current Microbiology Applied Sciences* **6**: 573–77.
- Sambrook J and Russell D. 2001. Molecular cloning: a laboratory manual, 3rd edn. Cold Spring Harbor Laboratory Press, New York.
- Sharma R, Pandey A K, Singh LV, 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**: 436–40
- Singh S, Mishra A K, Vohra V, Raja K N, Sing Y, Singh K M, Ganguly I and Arora R. 2017. Microsatellite based genetic diversity estimation in Kajali sheep and its phylogenetic relationship with other indigenous sheep breeds. *Indian Journal of Animal Sciences* **87**:1097–101.
- Twentieth Livestock Census, Department of Animal Husbandry and Dairying, Ministry of Fisheries, Animal Husbandry and Dairying.
- Yadav D K, Arora R and Jain A. 2017. Classification and conservation priority of five Deccani sheep ecotypes of Maharashtra, India. *PLoS One* **12**(9): e0184691.
- Yeh F C, Yang R C and Boyle T. 1999. POPGENE Version 1.32: Microsoft window-based freeware for population genetics analysis, University of Alberta, Edmonton.