

Breed characteristics and genetic variability in Changthangi goats

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ABSTRACT

Changthangi, a Pashmina yielding goat breed of India, is found at high altitudes ranging from 3000 to 6000 m above mean sea level in the trans Himalayan mountain range bordering Tibet. It is more localized in Changthang sub division of Ladakh region of Jammu and Kashmir. The study was done to know the genetic variability within breed. Genomic DNA isolated from randomly collected blood samples was amplified using standard PCR conditions before analyzing on an automatic DNA sequencer. A battery of 12 microsatellite markers was used for genetic characterization. The data thus generated were analysed to estimate the allele frequencies, allele number, heterozygosity, poly information content and inbreeding coefficient. The observed number of alleles varied from 5 (OarJMP 29) to 19 (OarFCB304) with a mean of 10.4 ± 3.91 . The effective number of alleles varied from 1.56 (OarJMP29) to 8.92 (OarFCB304) with a mean 4.59 ± 2.07 . The value of Shannon information index (I) ranges from 0.774 (OarJMP29) to 2.487 (OarFCB304) whereas poly information contents range from 0.343 (OarJMP29) to 0.846 (OarFCB304). The high value of I and PIC indicated the suitability of markers for studying the genetic variability in goat species. Observed heterozygosity ranged from 0.2368 (OarJMP29) to 0.9783 (OarFCB304). The mean observed heterozygosity was 0.3979. The expected heterozygosity was highest (0.8880) for locus Oarfeb 304 and lowest (0.3618) for locus OarJMP29. Nine of the 12 loci showed the positive inbreeding coefficient. Among the positive values the F_{is} varied from 0.0527 (ILSTS008) to 0.6491 (OMHC1) with a mean of 0.1773 however, ILST044, ILSTS002, OarFCB304 exhibited the negative values corresponding to 0.0471, 0.0855 and 0.1016 respectively. The L shaped curve obtained indicated that Changthangi population has not undergone any recent bottleneck. The study indicated that Changthangi goats exhibited substantial amount of genetic variation as reflected from the heterozygosity and number of alleles per locus. Avoiding unplanned and indiscriminate mating between individuals can increase the heterozygosity which can be exploited in the improvement of productivity of this breed.

Key words: Breed, Changthangi, Genetic variability, Pashmina

Changthangi and Chegu are 2 Pashmina yielding goat breeds of India (Acharya 1982) which are found at high altitudes in the trans Himalayan mountain range bordering Tibet. The pashmina is a silky undercoat of the long hairs. Changthangi also called Changra are localized in Changthang sub division of Ladakh region of Jammu and Kashmir. These animals are reared by Changpa, a nomadic tribe, and the breed is named after its natural habitat. Changthangi breed of goat is among the rare species inhabiting the high altitudes ranging from 3000 to 6000 m above mean sea level. The reports available on the genetic make up and diversity of Changthangi goats is scanty. In view of its existence under harsh climatic conditions and performing to yield a wonderful product like Pashmina of great export value for the country,

the breed is selected for knowing the genetic variability within the breed.

MATERIALS AND METHODS

Blood samples collected at random from 48 animals in the breeding tract were available in the laboratory for this study. Genomic DNA was isolated following the standard phenol/chloroform protocol (Sambrook *et al.* 1989). A battery of 12 microsatellite markers (Table 1) was used for genetic characterization of Changthangi breed. Polymerase chain reaction (PCR) was carried out using the same standardized parameters following touchdown- PCR protocol used by us for the amplification of genomic DNA (Verma *et al.* 2007a,b,c, Verma *et al.* 2009). After ensuring the amplification, the PCR products were run on automated DNA sequencer. The data thus generated were analysed using POPGENE (Yeh *et al.* 1999) to calculate the allele frequencies, allele number (Kimura and Crow 1964), and

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heterozygosity (Levene 1949, Nei 1973). Poly information content (PIC) values were estimated as per Botstein *et al.* (1980). Allelic richness was calculated with FSTAT (Goudet 1995).

RESULTS AND DISCUSSION

Native tract: The habitat of Changthangi breed of goat is the Changthang region of Leh district in Jammu and Kashmir (Fig. 1), which is one of the highest plateau in the world. The Ladakh region constitutes nearly one-third Jammu and Kashmir state of India. The plateau characterized by sandy soil and brown rocks among snow covered high range northwestern Himalayan Mountains. The region lies between 30° and 60° latitude and 75° to 81° longitude. The annual rain fall is 8 to 9 cm. The temperature in summer reaches as high as 35°C and in winter drops to -50°C. The important pasture species found in the area are grasses such as *Poa pratensis*, *Festuca rubra*, *Bromus macrostachy*, *Elymus sibiricus*, *Agropyron lange-aristatum* etc., legumes such as *Astragalus tribulipolius*, *Oxytropis micropylla*, *Cicer mycrophylum* etc. and bushes such as *Cargana vesicolor*, *Myricaria germonica*, *Myricaria prostrate* (Mir 2000).

Morphological characteristics and performance: Changthangi goats are medium sized animals having a coat of long hair. Average body measurements of Changthangi goats are given in Table 1. Body colour of Changthangi goats varies from white to light brown and nearly whole body is covered with long hair (Fig. 1). The hair are long and lusty



Fig 1. A Changthangi male goat

making a guard for the animal against extremely low temperature. The undercoat of long hair is called Pashmina. Animals of both sexes are generally horned and have beard. The horns are large, twisted, directed upward outward and backward. The animals particularly males have a prominent tuft of hair on the forehead. The head is small and triangular held high above back line with a strong and well set neck. The ears are small, erected and stumpy. The goats can walk long distances in search of pasture grasses and herbs. Females can mature at the age of 6 months.

The mean birth weight ranges from 1.82 kg in female (Darokhan and Tomar 1983a) to 2.25 kg in male (Patil 1992). The season of kidding had hardly any effect on the birth weight but age of dam at the time of kidding had significant effect on the growth of kid (Misra *et al.* 1998). Kumar (2000) reported that age at first tugging, age at first kidding, gestation period, kidding interval and litter size of Changthangi goat as 668 days, 830 days, 152 days, 397 days and single respectively. A doe yields about 200–300 ml milk/day, which is used for feeding the kids but after weaning the milk is used for home consumption. The yield and quality of pashmina depends upon the size of animal and differ from place to place. Male produces more pashmina than females of the same age group. Similarly castrated males produce more pashmina than the breeding males. The pashmina quality and quantity is influenced by sex, age, body weight, availability of nutrients, climatic conditions, ratio of secondary follicles and colour of pashmina (Wani *et al.* 2004). The pashmina fibre characteristics were reported by Drokhan (1983b), Acharya and Sharma (1980), Patil (1992), Misra *et al.* (1998) and Bhattacharya *et al.* (2000).

Genetic variability: Among the various molecular genetic markers microsatellite are considered to be the most powerful genetic markers owing to their highly polymorphic nature. The studies were carried out using a battery of 12 FAO recommended microsatellite markers. These microsatellites have dinucleotides type of repeats and size of their nucleotide sequences ranged between 105 and 195 (Table 2). These microsatellites are not species/breed specific but the studies conducted at NBAGR, on other goat breeds, showed the suitability of these markers for this species also. The genetic variation was estimated in terms of allele number, allelic frequency, heterozygosity, Shannon information index,

Table 1. Body weights and biometry of Changthangi goats at different ages

Age group	Sex	Body weight (kg)	Body length (cm)	Heart girth (cm)	Height at wither (cm)	Reference
Birth	Male	2.25	21.18	29.53	26.64	Patil (1992), Drokhan and Tomar (1983a)
	Female	2.13	20.53	28.45	25.72	
2 years	Male	25.50	51.20	65.00	55.60	Patil, (1982), Misra <i>et al.</i> (1998)
	Female	22.07	49.00	54.10	49.10	
Adult	Male	31.02	52.40	65.20	51.60	Acharya (1992), Patil (1992)
	Female	25.72	49.80	63.00	49.00	

Table 2. Microsatellite markers, their sequence, type of repeat, Accession number and chromosomal location

Primer	Sequences	Repeats	Dye	Accession No.	Chromo No.
ST008	F- gaatcatggatttctgggg R- tagcagtgagtgaggtggc	(CA) ₁₂	Fam	L23483	167–195
ILSTS059	F- gctgaacaatgtgatgttcagg R- gggacaatactgtcttagatgtctgc	(CA) _{4(GT)2}	Fam	L37266	105–135
ETH225	F- gatcaccttgccactatttct R- acatgacagccagctgcttact	(CA) ₁₈	Vic	Z14043	146–160
ILST044	F- agtcacccaaaagtaactgg R- acatgttgattcccaagtgc	(GT) ₂₀	Ned	L37259	145–177
ILSTS002	F- tctatacacatgtgtgtgc R- cttagggggtgaagtgcacg	(CA) ₁₇	Vic	L23479	113–135
OarFCB304	F- ccctaggagctttcaataaagaatcgg R- cgctgctgtcaactgggtcagg	(CT) ₁₁ (CA) ₁₅	Fam	L01535	119–169
OarFCB48	F- gagtttagtacaaggatgacagaggcac R- gactctagaggatgcgc	(GT) ₁₀	Vic	M82875	149–181
OarHH64	F- cgttcctcactaggaaagtatatatgc R- cactctattgtaagaattgaatgaatgagagc	–	Pet	212a	120–138
OarJMP29	F- gtatacacgtggacaccgcttgtac R- gaagtggcaagattcagagggaag	(CA) ₂₁	Ned	U30893	120–140
ILSTS005	F- ggaagcaatgaaatctatagcc R- tgttctgtgagtttgaagc	(nn) ₃₉	Vic	L23481	174–190
ILSTS019	F- aaggagacctcatgtagaagc R- acttttggaccctgtagtgc	(TG) ₁₀	Fam	L23492	142–162
OMHC1	F- atctggtgggctacagtcctatg R- gcaatgcttctaattctgaggaa	–	Ned	228b	179–209

polymorphic information content and f-estimates etc. The values estimated for these parameters are given in Tables 3, 4.

Allele number: Since the DNA sample was drawn at random, number of alleles observed in the samples for a given marker is supposed to represent the number of alleles in the whole population for that particular locus. The observed number of alleles varied from 5 (OarJMP 29) to 19 (Oar FCB304) with a mean of 10.4 ± 3.91 . The effective number

of alleles is the reciprocal of sum of the square of allele frequencies and varied from 1.56 (OarJMP29) to 8.92 (OarFCB304) with a mean 4.59 ± 2.07 . The effective number of alleles is less than the half of observed number for each locus. This may be due to the fact that large number of alleles in respect of each locus is present with a very low frequency. Similar observation was made in other breeds of goats by Kumar *et al.* (2005, 2006) and Aggarwal (2006).

Shannon's information index (I) and polymorphic

Table 3. Allele number, Shannon's information index and PIC

S.N.	Locus	Sample size	Observed number of alleles	Expected number of alleles	Shannon's information index	PIC
1.	ILST008	96	6	2.5153	1.1935	0.5617
2.	ILSTS059	82	8	2.8063	1.4349	0.6166
3.	ETH225	90	10	4.8854	1.7934	0.7681
4.	ILST044	96	15	4.0996	1.8703	0.7266
5.	ILSTS002	82	10	4.6825	1.8657	0.7653
6.	OarFCB304	92	19	8.9283	2.4869	0.8463
7.	OarFCB48	92	14	6.8039	2.1852	0.8322
8.	OarHH64	96	9	3.6398	1.5388	0.6845
9.	OarJMP29	76	5	1.5670	0.7745	0.3433
10.	ILSTS005	92	9	4.2447	1.7152	0.7355
11.	ILSTS019	92	9	4.0228	1.6133	0.713
12.	OMHC1	40	11	6.8966	2.1356	0.839
	Mean	86	10.41	4.5910	1.7173	
	±SE		3.9187	2.0747	0.4596	

Table 4. Homozygosities and heterozygosities

Locus	Sample size	Ob. Het.	Exp.Het.	Nei	Av. Het.	Fis
ILST008	96	0.2708	0.6088	0.6024	0.6024	0.5504
ILSTS059	82	0.6098	0.6516	0.6437	0.6437	0.0527
ETH225	90	0.7333	0.8042	0.7953	0.7953	0.0779
ILST044	96	0.7917	0.7640	0.7561	0.7561	-0.0471
ILSTS002	82	0.8537	0.7961	0.7864	0.7864	-0.0855
OarFCB304	92	0.9783	0.8978	0.8880	0.8880	-0.1016
OarFCB48	92	0.5000	0.8624	0.8530	0.8530	0.4139
OarHH64	96	0.6250	0.7329	0.7253	0.7253	0.1382
OarJMP29	76	0.2368	0.3667	0.3618	0.3618	0.3455
ILSTS005	92	0.6304	0.7728	0.7644	0.7644	0.1753
ILSTS019	92	0.6957	0.7597	0.7514	0.7514	0.0742
OMHC1	40	0.3000	0.8769	0.8550	0.8550	0.6491
Mean	86	0.6021	0.7412	0.7319	0.7319	0.1773
S.E		0.2360	0.1453	0.1429	0.1429	

information contents (PIC): The Shannon's information index represents the relative abundance of genetic information of a specific locus to the total information available of the loci. The value of I ranges from 0.774 (OarJMP29) to 2.487 (OarFCB304) whereas PIC ranges from 0.343 (OarJMP29) to 0.846 (OarFCB304). The high value of I and PIC indicated the suitability of markers for studying the genetic variability in goat species.

Heterozygosity: Observed heterozygosity ranged from 0.2368 (OarJMP29) to 0.9783 (OarFCB304). The mean observed heterozygosity was 0.3979. The expected heterozygosity, which is a function of alleles and its frequency, was highest (0.8880) for locus Oarfeb 304 and lowest (0.3618) for locus OarJMP 29. The Changthangi goats exhibited substantial amount of genetic variation as reflected from the heterozygosity and number of alleles per locus. The average genetic variation in these goats is comparable to that observed in Black Bengal and Chegu goats (Behl *et al.* 2003); Jakhrana goats (Kumar *et al.* 2005, Verma *et al.* 2007b); Marwari goats (Kumar *et al.* 2005, Dixit *et al.* 2007); Sirohi goats (Verma *et al.* 2007a); Attappady goats, Mehsana goats (Aggarwal *et al.* 2006); Kutchi goats (Dixit *et al.* 2006); Gohilwadi (Verma *et al.* 2007c) and Malabari (Verma *et al.* 2009). Gannai and Yadav (2001) reported heterozygosity ranging from 0.45 to 0.54 in Barbari, Jamnapari and Sirohi goats. Sharma *et al.* (2006) reported comparatively low genetic variation in Barbari and Beetal goats.

F-statistics: The F statistics was applied to all the 12 studied loci to know the extent of inbreeding in the population and the values are presented in Table 4. The Fis values provide the non-random union of gametes in the population, i.e. the mating among the individuals in the population which are related more than the average relationship. More the value of Fis, more the inbreeding in the population. The negative values of Fis the point towards outbreeding i.e. mating of individuals who are less related than the average relationship

of the population. Nine of the 12 loci showed the positive inbreeding coefficient. Among the positive values the Fis varied from 0.0527 (ILSTS008) to 0.6491 (OMHC1) with a mean of 0.1773 however, ILST044, ILSTS002, OarFCB304 exhibited the negative values corresponding to 0.0471, 0.0855 and 0.1016 respectively (Table 4). The 9 loci exhibiting homozygosity or heterozygotic deficiency showed deviation from Hardy Weinberg equilibrium. Significant heterozygotic deficiency was also reported by others (Barker *et al.* 2001, Kumar *et al.* 2005, Luikart *et al.* 1999) in goats.

The heterozygotic deficiency observed in Changthangi goat population may be due to one or more of the following reasons: segregation of non amplifying (null) alleles, Wahlund effects, and/or inbreeding. Distinguishing among these generally is difficult (Christiansen *et al.* 1974). However, nulls were most unlikely to be segregating at all loci studied. Wahlund effects (localities with known sub population) may not account for Changthangi goat population as it is isolated from other goat breeds and inhabit the difficult areas where migration of other goat breeds is difficult. Therefore, it seems that there is inbreeding in the population resulting from uncontrolled and indiscriminate mating leading to small population sizes, breeding between relatives and consequent genetic drift. Further lack of sufficient number of breeding bucks and use of same limited number of bucks in the population will increase the inbreeding.

Mode shift analysis: The microsatellite alleles were organized into 10 classes, which permit checking whether the scattering followed the normal L-shaped curve where alleles with low frequencies are numerous. The curve drawn for Changthangi population is shown in Fig.2. The L shaped curve thus obtained indicated that Changthangi population has not undergone any recent bottleneck.

The study indicated that Changthangi goats exhibited substantial amount of genetic variation as reflected from the

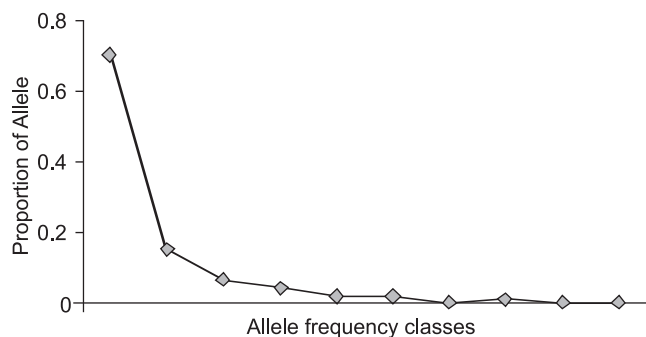


Fig 2. L-shaped mode-shift graph showing lack of bottle neck.

heterozygosity and number of alleles per locus. Avoiding unplanned and indiscriminate mating between individuals can increase the heterozygosity, which can be exploited in the improvement of productivity of this breed.

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