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Population genetics analysis of Mehsana goat based on microsatellite markers

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Genetic variation at 25 microsatellite loci was examined in Mehsana goats of Gujarat, which contribute significantly to the economy of small farmers by providing milk and meat. Estimation of effective number of alleles and gene diversity frequently observed in microsatellite markers revealed substantial genetic variation. The mean number of observed alleles per microsatellite marker was 12.28 and that of effective alleles was 6.23. The average observed and expected heterozygosity values were 0.652 and 0.765 respectively. The mean polymorphic information content value (0.724) further reflected high level of polymorphism across the loci. Within-population inbreeding estimate (F_{is} = 0.156) showed moderate level of inbreeding, which warranted adoption of appropriate breeding strategies under field conditions. The high level of genetic variability, however, suggested the scope for further genetic improvement of Mehsana goats. The information generated in this work may be utilized for studying differentiation and relationships among different Indian goat breeds.

Keywords: Genetic variation, Mehsana goats, microsatellite loci, polymorphism.

GOATS comprise one of the most important domestic livestock species in India and play an important role in the livelihood of a large proportion of small and marginal farmers and landless labourers. Since the goat provides a good source of meat, milk, fibre and skin, it is popularly known as the 'poor man's cow'. Goat diversity in India is reflected by 20 descript breeds[1] distributed throughout the country, which have evolved through natural selection for adaptation to specific agro-ecological conditions, ranging from the high altitude of the Himalayas to the deserts of Rajasthan and humid coastal areas of India. Most of these breeds are well adapted to the harsh climate, long migration, tropical diseases, poor nutrition and shortage of drinking water. However, indiscriminate crossbreeding, uncontrolled intermixing and geographical overlap are leading to the endangerment of breed purity, and potentially important caprine genetic material is being put to risk. Hence conservation of indigenous goat germplasm has been recognized as a task of national concern. Genetic characterization of native breeds of a species is a first step in prioritization of breeds for conservation. Here, we present population genetics analysis of the Mehsana goat, a well-accepted and widely distributed breed in North Gujarat. Mehsana goats are reared and maintained under extensive production system. They contribute significantly to rural economy by providing milk and meat products for rural as well as urban population.

Blood samples of 50 unrelated animals from 19 villages of three districts (Mehsana, Pattan, Banaskantha) from Gujarat were collected. Genomic DNA was isolated from these samples using a standard phenol : chloroform extraction method[2]. A battery of 25 microsatellite markers (Table 1) based on the guidelines of ISAG and FAO's DADIS programme were utilized to generate data in a panel of 48

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Table 1. Microsatellite markers, their sequences, type of repeat, size range, location and accession numbers

Locus	Primer sequence	Type of repeat	Size range	Chr. no.*	GenBank Acc. no.**
ILST008	gaatcatggattttctgggg tagcagtgagtgaggttggc	$(CA)_{12}$	167–195	14	L23483
ILSTS059	gctgaacaatgtgatatgttcagg gggacaatactgtcttagatgctgc	$(CA)_4(GT)_2$	105–135	13	L37266
ETH225	gatcaccttgccactatttcct acatgacagccagctgctact	$(CA)_{18}$	146–160	14	Z14043
ILST044	agtcacccaaaagtaactgg acatgttgtattccaagtgc	$(GT)_{20}$	145–177	Ann	L37259
ILSTS002	tctatacacatgtgctgtgc cttaggggtgaagtgacacg	$(CA)_{17}$	113–135	Ann	L23479
OarFCB304	ccctaggagctttcaataaagaatcgg cgctgctgtcaactgggtcaggg	$(CT)_{11}(CA)_{15}$	119–169	Ann	L01535
OarFCB48	gagttagtacaaggatgacaagaggcac gactctagaggatcgcaaagaaccag	$(GT)_{10}$	149–181	17	M82875
OarHH64	cgttccctcactatggaaagttatatatgc cactctattgtaagaatttgaatgagagc	–	120–138	4	212[a]
OarJMP29	gtatacacgtggacaccgctttgtac gaagtggcaagattcagaggggaag	$(CA)_{21}$	120–140	Ann	U30893
ILSTS005	ggaagcaatgaaatctatagcc tgttctgtgagtttgtaagc	$(nn)_{39}$	174–190	10	L23481
ILSTS019	aagggacctcatgtagaagc acttttggaccctgtagtgc	$(TG)_{10}$	142–162	Ann	L23492
OMHC1	atctggtgggctacagtccatg gcaatgctttctaaattctgaggaa	–	179–209	Ann	228[a]
ILSTS087	agcagacatgatgactcagc ctgcctcttttcttgagagc	$(CA)_{14}$	142–164	Ann	L37279
ILSTS30	ctgcagttctgcatatgtgg cttagacaacaggggtttgg	$(CA)_{13}$	159–179	2	L37212
ILSTS34	aagggtctaagtccactggc gacctggtttagcagagagc	$(GT)_{29}$	153–185	5	L37254
ILSTS033	tattagagtggctcagtgcc atgcagacagttttagaggg	$(CA)_{12}$	151–187	12	L37213
ILSTS049	caattttcttgtctctcccc gctgaatcttgtcaaacagg	$(CA)_{26}$	160–184	11	L37261
ILSTS065	gctgcaaagagttgaacacc aactattacaggaggctccc	$(CA)_{22}$	105–135	24	L37269
ILSTSO58	gccttactaccatttccagc catcctgactttggctgtgg	$(GT)_{15}$	136–188	17	L37225
ILSTSO29	tgttttgatggaacacagcc tggatttagaccagggttgg	$(CA)_{19}$	148–191	3	L37252
RM088	gatcctcttctgggaaaaagagac cctgttgaagtgaaccttcagaa	$(CA)_{14}$	109–147	4	U10392
ILSTS022	agtctgaaggcctgagaacc cttacagtccttggggttgc	$(GT)_{21}$	186–202	Ann	L37208
OarAE129	aatccagtgtgtgaaagactaatccag gtagatcaagatatagaatatttttcaacacc	$(CA)_{14}$	130–175	7	L11051
ILSTS082	ttcgttcctcatagtgctgg agaggattacaccaatcacc	$(GT)_{17}$	100–136	2	L37236
RM4	cagcaaaatatcagcaaacct ccacctgggaaggccttta	$(CA)_{13}$	104–127	15	U32910

*Chr. no., Chromosome number; **Acc. no., Accession number; [a]Accession number of Arkdb database (http://www.thearkdb.org); Ann, Anonymous microsatellite from other species.

animals. Each forward primer was tagged on the 5′-end with one out of the four dyes (FAM, PET, VIC, NED), as supplied by Applied Biosystems, UK.

PCR was carried out using 50–100 ng genomic DNA in a 25 µl reaction volume on PTC-200 PCR machine (M J Research). The reaction mixture consisted of 200 µM each of dATP, dCTP, dGTP and dTTP, 50 nM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.0 mM $MgCl_2$, 0.75 units Taq DNA polymerase and 50 ng of each primer. The 'touchdown' PCR protocol was used with initial denaturation of 95°C for 1 min, three cycles of 95°C for 45 s and 60°C for 1 min, three cycles of 95°C for 45 s and 57°C for 1 min, three cycles of 95°C for 45 s and 54°C for 1 min, three cycles of 95°C for 45 s and

Table 2. Number of alleles, heterozygosity, polymorphic information content (PIC) and inbreeding estimate (F_{is}) for each microsatellite locus and mean estimate of different parameters for Mehsana goat

Locus	Number of alleles		Heterozygosity*		Information index	PIC	F_{is}	P value
	Observed (n_a)	Effective (n_e)	Observed (H_o)	Expected (H_e)				
ILST008	3	1.1905	0.0571	0.1623	0.3471	0.1532	0.651	0.0040
ILSTS059	11	4.5224	0.7097	0.7916	1.8469	0.7492	0.105	0.1420
ETH225	5	1.4746	0.0909	0.3268	0.7210	0.3103	0.784	0.0020
ILSTS044	13	4.4627	0.7429	0.7872	1.9346	0.7528	0.051	0.2940
ILSTS002	16	9.2814	0.6757	0.9045	2.4582	0.8671	0.273	0.0020
OarFCB304	21	11.3155	0.9565	0.9216	2.6605	0.8358	–0.033	0.8800
OarFCB48	18	9.0621	0.7826	0.8994	2.4412	0.8676	0.133	0.0120
OarHH64F	12	8.5069	0.6857	0.8952	2.2612	0.8697	0.235	0.0020
OarJMP29	11	6.6494	0.1562	0.8631	2.0764	0.8249	0.821	0.0020
ILSTS005	9	4.1523	0.4043	0.7673	1.6937	0.7252	0.491	0.0020
ILSTS019	9	4.0960	0.7292	0.7638	1.6138	0.7155	0.062	0.2420
OMHC1	19	11.9548	0.9130	0.9264	2.6871	0.8524	0.019	0.4100
ILSTS087	13	9.4426	0.7292	0.9035	2.3755	0.8679	0.191	0.0020
ILSTS30	16	9.7060	0.7442	0.9075	2.4733	0.8695	0.182	0.0060
ILSTS34	8	4.2353	0.6667	0.7719	1.6865	0.7348	0.120	0.0800
ILSTS033	8	2.2916	0.6552	0.5735	1.2699	0.539	–0.145	0.9780
ILSTS049	13	7.7709	0.8571	0.8818	2.2948	0.8401	0.033	0.3540
ILSTS065	4	1.8416	0.3043	0.4620	0.8340	0.4126	0.344	0.0020
ILSTS058	24	12.6247	0.8958	0.9305	2.8083	0.8785	0.040	0.2220
ILSTS029	9	2.3704	0.6042	0.5842	1.1931	0.5352	–0.035	0.7240
RM088	17	5.2814	0.9048	0.8204	2.1496	0.7754	–0.094	0.9760
ILSTS022	7	4.3549	0.5854	0.7799	1.6023	0.7341	0.252	0.0040
OarAE129	13	4.3678	0.9583	0.7792	1.8301	0.7373	–0.253	1.0000
ILSTS082	18	9.0134	0.8049	0.9000	2.4706	0.8574	0.135	0.0200
RM4	10	5.8701	0.7083	0.8384	1.9534	0.8082	0.165	0.0160
Mean	12.28	6.2336	0.6529	0.7657	1.9073	0.7245	0.156	0.002
St. Dev.	5.35	3.4115	0.2583	0.1979	0.6452			

*Values of observed and expected heterozygosity differ significantly ($P \leq 0.05$) with each other according to Wilcoxon matched pairs test.

51°C for 1 min, and 20 cycles of 95°C for 45 s and 48°C for 1 min. At the end of the reaction, 5.0 µl of stop dye (95% formamide, 0.25% bromophenol blue and 0.25% xylene cyanol) was added to terminate the reaction. Next 6 µl of PCR products was loaded onto a 2% agarose gel, electrophoresed and visualized over UV light after ethidium bromide staining to detect amplification.

Microsatellite genotyping was carried out using AVANT 3100 automated DNA sequencer (Applied Biosystems) with LIZ 500 as the internal lane standard. Data were collected and analysed using Gene Mapper Software (Version 3.0, Applied Biosystems).

For the 25 microsatellite loci analysed, observed and expected heterozygosity estimates were calculated according to Levene[3] and Nei[4], and as implemented in the POPGENE software[5]. The observed and effective number of alleles according to Kimura and Crow[6] was also calculated using the POPGENE software.

Tests for deviation from Hardy–Weinberg equilibrium were derived using the F statistics of FSTAT program. Tests for pairwise linkage (genotypic) disequilibrium among microsatellite loci were also done using FSTAT version 2.9.3, an updated[7] version of 1.2, for 25 microsatellite loci whose genotypes were determined directly. Polymorphism information content (PIC) was calculated according to Botstein *et al.*[8]. Finally, the bottleneck hypothesis was investigated[9] using BOTTLENECK 1.2.01. The program tests for departure from mutation drift equilibrium based on heterozygosity excess or deficiency, and requires only measurement of allele frequencies from 5 to 20 polymorphic loci in a sample of approximately 20–30 individuals. It compares heterozygosity expected (H_e) at Hardy–Weinberg equilibrium to the heterozygosity expected (H_{eq}) at mutation drift equilibrium in the same sample, that has the same size and the same number of alleles. The strict one-step stepwise mutation model (SMM)[10], the infinite allele model (IAM)[6] and two phase mutation model (TPM)[11] were used to calculate H_{eq}.

Different measures of genetic variation in Mehsana goat population are presented in Table 2. The number of alleles observed across the studied microsatellite loci varied from 3 (ILST008) to 24 (ILSTS058), with an overall mean of 12.28 ± 5.35. The observed number of alleles was more than the effective number of alleles, which ranged from 1.19 (ILST008) to 12.62 (ILSTS058). The average observed heterozygosity (0.65) was lower than the expected heterozygosity (0.76). The average expected gene diversity within the population ranged from 0.16 (ILST008) to 0.93 (ILSTS058). Shanon's information index and PIC showed that most of the loci were highly

Table 3. Test for mutation drift equilibrium at 25 microsatellite loci under three mutation models in Mehsana goat

	IAM		TPM		SMM	
Test	Expected	Observed	Expected	Observed	Expected	Observed
Sign test (number of loci with heterozygosity excess)						
	15.01	17	14.88	15	14.75	4**
Standardized difference test (T_2 values)						
	1.915*	–	–0.537	–	–8.748**	–
Wilcoxon test (probability of heterozygosity excess)						
	0.03335*	–	0.48946	–	0.99996	–

*$P \leq 0.05$, **$P \leq 0.01$.

IAM, Infinite allele model; TPM, Two-phase mutation model; SMM, Strict one-step stepwise mutation model.

informative indicating high polymorphism across the loci with an overall mean of 1.90 and 0.72 respectively, thus suggesting suitability of these markers for genetic diversity studies in goats.

The Mehsana goats had substantial genetic variation based on their gene diversity and average number of alleles per locus. The average genetic variation (0.652) in Mehsana goats was comparable with genetic variation found in Black Bengal (H_o = 0.69) and Chegu (H_o = 0.66) breeds of Indian goats[12], and was higher than in other indigenous goats (Barbari, Jamnapari, Sirohi and Marwari: H_o ranging from 0.45 to 0.54)[13,14].

Thirteen of the 25 loci studied showed significant heterozygote deficiency (positive deviation) in the Mehsana goat population as analysed by F-statistics (Table 2). Within-population inbreeding estimate (F_{is}) for the investigated loci was 0.156. Significant heterozygote deficiency has also been reported in some other studies of goats[14–16]. Heterozygote deficiency found in Marwari goat of Rajasthan[14] could be due to one or more of the following reasons: segregation of non-amplifying (null) alleles, Wahlund effects, and scoring biases or inbreeding; however distinguishing among these generally is difficult[17]. Moderate level of inbreeding (F_{is} = 0.156) observed in the present study suggests unplanned and indiscriminate breeding. This may be due to the fact that few bucks are used for the whole and nearby villages in the breeding region. Significant linkage disequilibrium could not be detected at any of the loci pairs located on the same chromosome after applying Bonferroni corrections in deriving the level of significance (P = 0.00017).

The sign test revealed substantial differences between the number of loci observed and expected with heterozygosity excess (Table 3). Four out of 25 studied microsatellite loci had hetrozygosity excess, while the remaining 21 showed significant ($P \leq 0.01$) heterozygosity deficiency under SSM. The dataset showed mutation drift equilibrium under IAM and TPM models.

The standardized difference and Wilcoxon test revealed significant heterozygosity excess under IAM and mutation drift equilibrium under TPM. Standardized difference test showed significant heterozygosity deficiency (T_2 = –8.748, $P \leq 0.01$) in Mehsana goat population under SMM, while Wilcoxon test revealed that the population had undergone recent bottleneck assuming the IAM. The mode shift test, however, revealed mutation drift equilibrium in this breed of goat.

All three tests used to study mutation drift equilibrium revealed significant heterozygosity deficiency in Mehsana goat population under SMM and mutation drift equilibrium under TPM. The mutation model for most of the loci is probably intermediate between the IAM and SMM[11]. It may be safely concluded that the Mehsana goat population had not faced any recent genetic bottleneck.

The result of this study suggests that there is substantial genetic variation and polymorphism across the studied loci in Mehsana goats. The population has moderate level of inbreeding. The study suggests scope for its further genetic improvement and to undertake appropriate breeding strategies to avoid inbreeding in the population.

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Two-species microbial consortium for growth promotion of *Cajanus cajan*

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We studied the interactions and the importance of a unique relationship in a plant growth promoting consortium comprising two species, *Burkholderia* sp. MSSP and *Sinorhizobium meliloti* PP3. They are rhizospheric isolates with abilities to produce indole-3-acetic acid (IAA) and solubilize inorganic phosphate. The organisms were grown as monospecies or mixed-species culture and studied for growth profile, IAA production and phosphate solubilization. *Burkholderia* sp. MSSP was marked with green fluorescent protein reporter gene to monitor growth in mixed-species culture. The growth rate of PP3 increased in mixed species culture, while that of MSSP remained unaffected. IAA production increased about 50% in mixed-species culture, compared to maximum IAA released in individual trials. The amount of phosphate solubilized was not affected. The two strains were tested on *Cajanus cajan* for their plant growth promoting activities in sterile soil. Inoculation of either MSSP or PP3 resulted in significant increase in seedling length and weight. In accordance with the findings of *in vitro* experiments, exceptional increase in seedling growth was recorded in mixed-species, co-inoculated consortium.

Keywords: *Burkholderia* sp., *Cajanus cajan*, growth promotion, microbial consortium, *Sinorhizobium meliloti*.

BACTERIA live in consortia bound to surfaces such as in biofilms, flocs or granules. Under these conditions the bacteria are positioned in a heterogeneous environment. It is increasingly apparent that in nature, bacteria function less as individuals and more as coherent groups that are able to inherent multiple ecological niches[1]. Populations of bacteria have functional roles within communities that permit their survival. Distinct microbial populations in rhizosphere frequently interact with each other. Syntrophic relationships between different organisms have been demonstrated in several microbial ecosystems. Therefore, mixed inoculants (combination of microorganisms) that interact synergistically are currently being devised, which yield better and quick results[2]. Recently, a microbial consortium for plant growth promotion was suggested[3]. It has been suggested that development of plant growth promoting consortium (PGPC), could be a feasible strategy for increased activity and better viability of plant growth promoting rhizobacteria (PGPR). When these strains are made into an inoculum consortium, each of the constituent strains of the consortium not only out-compete with the others for rhizospheric establishments, but complement functionally for plant growth promotion[4]. Here, we describe the relationship between two distantly related isolates, *Burkholderia* sp. MSSP and *Sinorhizobium meliloti* PP3. We discovered that in combination they promote growth of host plants because of increased indole-3-acetic acid (IAA) production. IAA is a member of the auxin family of the phytohormones that influence many cellular functions in plants and promote plant growth even without concomitant nitrogen fixation, or with heavy nitrogen fertilizers[5]. Phosphate (P) solubilization is another mechanism by which unavailable, immobilized, precipitated phosphorus of applied fertilizers is brought back into the medium by the action of mineral and organic acids produced by bacteria[6]. Both isolates had the ability to solubilize inorganic P and hence P solubilization in mixed-culture was also determined. Both the strains were studied in conjunction to each other as 'two-species' PGPC, for IAA production, P solubilization and effectiveness of this combination for growth promotion of *Cajanus cajan*. Green fluorescent protein (GFP)-based reporter system was utilized to monitor growth in the present study.

Several PGPR were tested in different combinations to observe their effect on the growth of *C. cajan* (data not given). One of the combinations consisting of *Burkholderia* sp. MSSP and *S. meliloti* PP3, was found to enhance growth in pot conditions significantly compared to non-bacterized control or single-species trials (described later). These two bacterial strains were selected for the present study. These

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