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Molecular Characterization of Mali Pigs of Tripura Using Microsatellite Markers

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Abstract: The genetic diversity and population structure of Mali pigs of Tripura was investigated using a set of 19 microsatellite markers recommended by Food and Agricultural Organization of United Nations (FAO) for Swine. All the studied loci were highly informative. The number of observed alleles (N_a) detected ranged from 3 to 11, with an overall mean of 5.63±0.663. In total 121 alleles were observed across the investigated loci. The effective number of alleles (N_e) ranged from 1.172 to 6.502 with a mean of 3.16±0.328. The PIC value ranged from 0.141 to 0.794 with the overall mean of 0.53±0.266. The overall means for observed (H_O) and expected (H_e) heterozygosities were 0.38±0.052 and 0.57±0.059 respectively. The within breed inbreeding estimate indicated heterozygosity shortage of 0.290. The Hardy-Weinberg equilibrium test revealed that 14 out of 19 loci deviated from equilibrium. Shannon's information index (I), was sufficiently high with a mean of 1.18±0.133. The bottleneck analysis revealed that population has not undergone any recent reduction.

Key words: Mali Pig % Heterozygosity % Microsatellites % Polymorphic Information Content (PIC)

INTRODUCTION

Tripura is the third smallest state of India. The only available pig genetic group in the state of Tripura is known as 'Mali'. The native germplasm under study, Mali pig is distributed in almost all the districts of Tripura with the ability to survive in low input systems. These pigs are black in colour with compact body covered with thick coarse long hair, short legs and a long tail (Fig. 1). The face is narrow with an upwardly curved snout and possesses medium size hindquarters and drooping rumps [1]. The average litter size at birth of Mali pigs is 7.34±0.71 [2].

Several molecular techniques have now emerged for the detection of variations at the DNA level, which include Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and Microsatellites. Among these, microsatellites are highly polymorphic with co-dominant inheritance, abundant in nature and distributed throughout the genome. It has been established that microsatellite markers are suitable for description of breeds/genetic groups. Microsatellite markers are also used to identify genes associated with important economic traits such as meat, milk etc.

To date no studies have been documented on this pig population to understand the population diversity and structure at molecular level. The present study has been planned to examine the genetic diversity and population structure of Mali pigs using 19 polymorphic microsatellite markers.

MATERIALS AND METHODS

Sampling and DNA Isolation: A total of 40 blood samples were collected randomly into vacutainer tubes (4 ml) containing EDTA (7.2 mg) from genetically unrelated individuals of Mali pigs from their native breeding tract in Tripura state. The samples were transported to the laboratory on ice and stored at 4°C. Genomic DNA was extracted by standard phenol-chloroform method [3] with few modifications.

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Fig. 1: Typical Mali pigs in organized farm (A) Sow (B) Boar (C) Sow with piglets and (D) Sow with drooping rumps

Table 1: Outline of markers used in the present study

Locus	Gene bank Accession Number	**Ch. No.	Primer sequences (5' 6 3')	Dye	T_a (°C)	Size range (bp)	
						In source reference	In present study
TNFB	X54859	7	F: ATCGCATCTGGTCAGCCACCAAGA				
			R: TTAGGAGGATTTTGCAACAACCCA	FAM	60	142-203	143-173
SO386	U78012	11	F: GAACTCCTGGGTCTTATTTTCTA				
			R: GTCAAAAATCTTTTATCTCCAACAGTAT	VIC	48	164-182	167-181
SW24	AF235245	17	F: CTTTGGGTGGAGTGTGTGC				
			R: ATCCAAATGCTGCAAGCG	VIC	58	92 -112	94-110
SO355	L29049	15	F: TCTGGCTCCTACACTCCTTCTTGATG				
			R: GTTTGGGTGGGTGCTGAAAAATAGGA	PET	55	247-273	243-265
SW353	AF235282	6	F: CACCCCATGCCTGAATACTG				
			R: ATGTGAAGACTCATGCTTGGG	NED	58	140-162	145-161
SO107	Ann	4	F: CAAGGATGCCTGTAACTGGTGCAG				
			R: TCCTTAAGGCCTCGTAGGATCTGT	NED	55	140-162	193-201
SO228	Ann	Ann	F: GGCATAGGCTGGCAGCAACA				
			R: AGCCCACCTCATCTTATCTACACT	FAM	55	231-245	223-245
SW72	AF235346	3	F: ATCAGAACAGTGCGCCGT				
			R: TTTGAAAATGGGGTGTTTCC	PET	58	100-116	100-116
SW122	AF235206	6	F: TTGTCTTTTTATTTTGCTTTTGG				
			R: CAAAAAAAGGCAAAAGATTGACA	NED	58	110-132	100-130
SO008	Ann	Ann	F: GAGGCAGTGTGTTCTATTCA				
			R: GCCATGTGTAAAGTGTTGCT	FAM	58	218-242	236-242
SO070	Ann	Ann	F: GGCGAGCATTTCATTCACAG				
			R: GAGCAAACAGCATCGTGAGC	FAM	62	290-296	273-293
SW957	AF235415	12	F: AGGAAGTGAGCTCAGAAAGTGC				
			R: ATGGACAACTTGGTTTTCC	VIC	58	112-157	112-156
SO227	L29196	Ann	F: GATCCATTTATAATTTTAGCACAAAGT				
			R: GCATGGTGTGATGCTATGTCAAGC	VIC	55	233-253	229-253
SW911	AF225106	9	F:CTCAGTTCTTTGGGACTGAACC				
			R:CATCTGTGGAAAAAAAAAGCC	PET	60	153-177	153-177
SO086	Ann	Ann	F: GCACAGTCTATTGATACTGGCGTC				
			R: CTGAGAACTTCCATATGCTCCTGG	NED	62	180-196	162-184
SO90	M95002	12	F: CCAAGACTGCCTTGTAGGTGAATA				
			R: GCTATCAAGTATTGTACCATTAGG	FAM	58	244-251	244-251
CGA	D00768	1	F: ATAGACATTATGTCCGTTGCTGAG				
			R: GAACTTTCACATCCCTAAGGTCGT	VIC	62	250-320	250-320
SW352	AF235281	7	F: CGTTAAGCCACTTTCTGTGG				
			R: AAGGGGGAGGGCGAGGGA	VIC	55	110-115	110-118
IGFI	Ann	5	F: GCTTGGATGGACCATGTTG				
			R: CACTTGAGGGGCAAATGATT	NED	58	197-209	197-209

**, Chromosome number; T_a, Annealing temperature; bp, Base pair.

Amplification of Microsatellite Regions: All the 19 well-characterized microsatellite markers (Table 1) were selected from the list recommended by Food and Agricultural Organization of United Nations (FAO) for Swine [4] based on their level of polymorphism, allele size range and reliability of allele calling. The forward primer of each of the marker was fluorescently labeled with either FAM, NED, PET or VIC dye. All the microsatellite markers were amplified using thermal cycler (Applied Biosystem, Model #: 9902) under single locus PCR conditions to evaluate their performance in the multiplex and accordingly multiplex panels were prepared for fluorescence genotyping. The PCR reaction mixture (15 µl) containing 20-50 ng of template DNA; 1.5 mM $MgCl_2$; 5 pM each of forward and reverse primers; 1 unit of taq DNA polymerase and 200 mM dNTPs was prepared. Amplification was carried out with initial denaturation at 95°C for 2 min followed by 30 cycles of denaturation (95°C for 30 sec), annealing (48°C to 62°C for 30 sec) and extension (72°C for 45 sec). After conformation of amplification on 2% agarose gel, the samples were processed for genotyping on an automated DNA Sequencer (ABI HITACHI 3500).

Statistical Analysis: The resulting data were analyzed using stranded software Gene Mapper^{TM} version 4.0 (Applied Biosystems Inc., California, USA) to generate genotype calls for each locus by using GS 500 (- 250) LIZ as size standard. The allele frequencies, effective number of alleles, test of Hardy-Weingberg equilibrium, observed and expected heterozygosity, F-statistics and Shanon's Information Index were calculated by using POPGENE v. 1.32 [5]. Nei's formula [6] was used to calculate polymorphic information content (PIC). The BOTTLENECK v. 1.2.03 [7] analysis was performed to know whether this pig population exhibiting a significant number of loci with excess of heterozygosity.

RESULTS

The results of genetic diversity in Mali pigs are presented in Table 2.The number of observed alleles (N_a) detected ranged from 3 (SO386, SO107 and SW355) to 11 (SO227 and SW122), with an overall mean of 5.63±0.663 and a total of 121 alleles were detected at these loci in the population. However, the effective number of alleles (N_e) ranged from 1.172 (at locus SO386) to 6.502 (at locus SO228) with a mean of 3.16±0.328. The PIC value in Mali pigs ranged from 0.1416 (SO386) to 0.7941 (CGA) with a mean of 0.53±0.266. The overall means for observed (H_O) and expected (H_e) heterozygosities were 0.38±0.052 and 0.57±0.059, respectively with the ranges of 0.154 (SO355) to 0.760 (SW911) and 0.147 (SO386) to 0.846 (SO228). The chi-square (P^2) test for Hardy-Weinberg equilibrium revealed that 14 out of 19 loci deviated from equilibrium.

Table 2: Microsatellite analysis in Mali pigs

		Parameters							
Panel	Locus	N_a	N_e	PIC	H_o	H_e	I	F_{IS}	HWE
Panel 1	SO386	3.000	1.172	0.1416	0.156	0.147	0.327	-0.063	0.230 ^{NS}
Panel 2	SW353	4.000	2.966	0.5971	0.276	0.663	1.170	0.584	54.011***
	TNFB	5.000	3.778	0.6925	0.464	0.735	1.447	0.369	51.661***
	SW024	4.000	2.765	0.5842	0.321	0.638	1.170	0.497	58.697***
	SO355	3.000	2.939	0.5855	0.154	0.660	1.088	0.767	32.877***
Panel 3	SO107	3.000	2.722	0.5611	0.690	0.633	1.050	-0.090	18.356***
	SO090	6.000	4.452	0.7378	0.406	0.775	1.563	0.476	43.859***
Panel 4	CGA	10.000	5.361	0.7941	0.188	0.813	1.957	0.770	173.724***
	SW072	6.000	3.606	0.6786	0.750	0.723	1.444	-0.038	20.665 ^{NS}
	SO228	10.000	6.502	0.829	0.625	0.846	2.048	0.261	79.650**
Panel 5	SO227	11.000	5.673	0.804	0.656	0.824	1.993	0.203	79.144*
	SW122	11.000	3.277	0.6729	0.469	0.695	1.657	0.325	82.385**
	SO008	8.000	2.090	0.4966	0.563	0.521	1.161	-0.079	9.351 ^{NS}
	SW957	5.000	2.014	0.4619	0.625	0.503	0.976	-0.242	4.532 ^{NS}
Panel 6	IGFI	7.000	3.670	0.6969	0.250	0.728	1.569	0.656	112.112***
	SO070	7.000	3.294	0.6453	0.321	0.696	1.381	0.538	31.656 ^{NS}
Panel 7	SW352	4.000	1.865	0.4329	0.346	0.464	0.905	0.254	52.171***
Panel 8	SW911	7.000	4.072	0.7141	0.760	0.754	1.544	-0.007	34.521*
Panel 9	SO086	7.000	4.401	0.7442	0.520	0.773	1.677	0.327	58.319***
Mean overall loci		5.63±0.663	3.16±0.328	0.53±0.266	0.38±0.052	0.57±0.059	1.18±0.133	0.290	

* Significant (P#0.05); **Highly significant (P#0.01); ^{NS} Not significant (P#0.05); N_a, Number of alleles; N_e, Effective number of alleles; PIC, Polymorphic information content; H_o, Observed Heterozygosity; H_e, Expected Heterozygosity; F_{IS}, Deficit or increase of heterozygotes, HWE, Hardy-Weinberg equilibrium; I, Shannon's Information Index.

Table 3: Bottleneck analysis in Mali pigs

	Sign rank test-Number of loci with heterozygosity excess				
Model	Expected	Observed	Probability	Standardized differences test-T2 values (probability)	Wilcoxon test-Probability of heterozygosity excess
IAM	11.19	12	0.44783	0.093 (0.46279)	0.22067
TPM	11.41	9	0.18477	-2.424 (0.00767)	0.77933
SMM	11.33	6	0.01256	-6.254 (0.00000)	0.98709

IAM - Infinite allele model; TPM - Two phase model; SMM - Stepwise mutation model

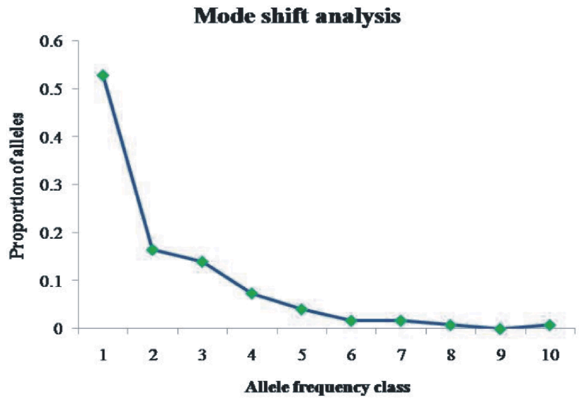

Fig. 2: Graphical representation of allele proportions and their contribution in Mali pig population

The within population heterozygotes deficiency (F_{IS}) observed at 13 loci were positive and 6 loci revealed negative with a mean of 0.29. Shannon's information index (I), which measures the level of diversity, was sufficiently high with a mean of 1.18.

Three mutation models namely, infinite allele model (IAM), two phase model (TPM), stepwise mutation model (SMM) were estimated using the programme Bottleneck (Table 3). The results indicated that Mali pig population is non-bottlenecked, i.e., it has not undergone any recent reduction in the effective population size and remained at mutation-drift equilibrium and no mode-shift was detected in the presentation investigation (Fig. 2).

DISCUSSION

All the 19 loci studied in Mali pigs were polymorphic in nature and the average expected heterozygosity i.e., gene diversity [8] was in the ranged from 0.147 to 0.846. The number and sizes of microsatellite alleles observed in this study fall within the range mentioned in the Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans of FAO.

The mean number of alleles observed (5.63) in the study is lower than the mean number reported for North Indian, Northeast Indian pig types (7.92 and 7.84) respectively [9] and Brazilian (8.96) pig breeds [10]. Zaman *et al*. [11] reported the mean observed alleles (N_a) of Ghungroo pig of North Bengal as 4.90±2.567 with a total of 103 alleles. The effective number of alleles observed (3.16) in the investigation is almost similar to that of Ghungroo pig 3.15±1.546 [11]. However, contrary to the present finding lower mean number of effective alleles were reported in Brazilian pig breeds viz., Landrace (2.70), Monterio (2.34), Moura (2.32), MS60 (2.56) and Piau (2.94) [10]. The low effective number of alleles than the observed number of alleles in the pig population under study may be due to very low frequency of most of the alleles at each locus and a very few alleles might have contributed the major part of the allelic frequency at each locus.

The PIC value in Mali pigs ranged from 0.1416 to 0.7941 which is corroborates with the mean PIC (0.49±0.171) in Meghalaya local pigs [12] and in Brazilian pig breeds mean PIC value of 0.655 have earlier been reported [10] using 28 different microsatellite markers, which is also comparable with present investigation. Most of the loci under study possessed high PIC values (above 0.50) signifying that these markers are highly informative for characterization of Mali pigs. The mean observed and expected heterozygosity (0.38 and 0.57) in the present study is lower than the mean number of observed and expected heterozygosity in Brazilian pig breeds (0.5841 and 0.685) [10]. The observed heterozygosity in Mali pigs is also lower than the South African domestic pigs viz., Landrace (0.522); Large White (0.584); Duroc (0.504); Namibia (0.518); Mozambique (0.609); Kolbroek (0.537) and Kune-Kune (0.508) [13]. However, the heterozygosities observed in the investigation are not in accordance with the values reported for microsatellites in Indian pig populations [9] and Chinese pig breeds [14,15].

The chi-square (P^2) test revealed that the deviation of 14 loci may be due to the genetic drift; non-random mating, non-amplifying alleles or the population might have been divided into a series of closely related or inbred family groups. The overall mean F_{IS} (0.29) observed in the present study indicating a 29% shortfall of heterozygosity in Mali pig population which may be attributed to several factors viz., the genetic drift; non-random mating, non-amplifying alleles or the population might have been divided into a series of closely related or inbred family groups.

Bottleneck analysis revealed that the population is non-bottlenecked where the mode-shift for the frequency distribution of alleles had a normal L-shaped curve stating that there was no recent reduction in the effective population size.

CONCLUSION

This investigation stands first in genetic characterization of Mali pigs using microsatellite markers and results revealed that the polymorphic nature of microsatellite loci screened in Mali pigs. The population has not undergone any reduction at least in the recent past. The shortfall of variability in this population is indicative of the loss of a valuable genetic diversity.

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