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Molecular characterization of Votho pigs from Nagaland using microsatellite markers

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The native germplasm of pig Votho, was predominantly distributed in Kohima, Peren and Phek districts of Nagaland with the ability to survive in low input systems. These pigs are having round compact body with small ears and short legs. The animals coat colour varies from gray to black and brown with bristle like hairs on the body. Because of its meat taste Votho pigs are considered as an important germplasm in Nagaland and adjoining states. To date no studies have been done on this pig population to understand the population diversity and structure at molecular level. The present study was planned to examine the genetic diversity and population structure of Votho pigs using 21 polymorphic microsatellite markers.

Sampling and DNA extraction: Blood samples (40) were collected randomly into vacutainers (4 ml) containing EDTA (7.2 mg) from genetically unrelated Votho pigs from their native breeding tract in Nagaland. The samples were transported to the laboratory on ice and stored at 4°C. Genomic DNA was extracted by standard phenol-chloroform method (Sambrook *et al.* 1989) with few modifications like using of DNAzol reagent instead of SDS and proteinase K.

Selection of microsatellite markers and amplification: All the 21 well-characterized microsatellite markers were selected from the list recommended by Food and Agriculture Organization of United Nations (FAO) for Swine (FAO 1998) 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 micro-satellite markers were amplified using thermal cycler 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.

Genotyping and data analysis: The resulting data were analyzed using stranded software Gene Mapper^{TM} v. 4.0 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 (Yeh *et al.* 1999). Polymorphic information content (PIC) was calculated according to Nei (1978). The BOTTLENECK v. 1.2.03 (Cornuet and Luikart 1996) was used to know whether this pig population exhibiting a significant number of loci with excess of heterozygosity.

The various parameters of genetic differentiation in Votho pig, such as allele number, effective number of allele, PIC, observed and expected heterozygosity, within-population inbreeding estimate (F_{IS}) and Shanon's information index are furnished in Table 1.

Most of the loci investigated were polymorphic in nature. The number of observed alleles (N_a) detected ranged from 2 (SO386 and SO107) to 10 (TNFB and IGFI), with an overall mean of 5.59±0.598 and a total of 122 alleles were observed at these loci in the population. The mean number of alleles observed (5.59) in the study is lower than the mean number reported for North Indian, North-east Indian pig types (7.92 and 7.84) respectively (Kaul *et al.* 2001) and Brazilian (8.96) pig breeds (Sollero *et al.* 2010). Zaman *et al.* (2013a) 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 (N_e) ranged from 1.054 (SO386) to 5.075 (SW072) with a mean of 3.02±0.284 in Votho pig is almost similar to that of

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Table 1. Microsatellite analysis in Votho pig

Panel	Locus	Parameters							
		N_a	N_e	PIC	H_o	H_e	I	F_{IS}	HWE
Panel 1	SW936	4	2.907	0.5886	0.727	0.656	1.166	–0.109	24.709***
	SO386	2	1.054	0.0499	0.053	0.051	0.122	–0.027	0.014 ^{NS}
Panel 2	SW353	3	1.925	0.4052	0.579	0.481	0.789	–0.205	22.678***
	TNFB	10	4.820	0.7704	0.458	0.793	1.869	0.422	104.079***
	SW024	3	1.135	0.1151	0.125	0.119	0.274	–0.051	0.107 ^{NS}
	SO355	3	2.057	0.4254	0.105	0.514	0.826	0.795	16.861***
Panel 3	SO107	2	1.117	0.0994	0.111	0.105	0.215	–0.059	0.062 ^{NS}
	SO090	5	4.341	0.7341	0.136	0.770	1.539	0.823	69.035***
Panel 4	CGA	9	3.767	0.6964	0.318	0.735	1.598	0.567	93.367***
	SW072	9	5.075	0.775	0.792	0.803	1.793	0.014	22.510 ^{NS}
	SO228	8	4.189	0.7359	0.458	0.761	1.726	0.398	110.519***
Panel 5	SO227	5	3.390	0.6623	0.000	0.705	1.383	1.000	80.000***
	SW122	6	4.590	0.748	0.417	0.782	1.622	0.467	64.269***
	SO008	5	2.318	0.5357	0.500	0.569	1.150	0.121	24.240**
	SW957	6	2.462	0.5116	0.208	0.594	1.092	0.649	24.648 ^{NS}
Panel 6	SO010	5	2.522	0.5506	0.625	0.604	1.147	–0.036	20.938*
	IGFI	10	3.433	0.6868	0.409	0.709	1.679	0.423	102.255***
	SO070	8	3.840	0.7129	0.737	0.740	1.657	0.004	39.321 ^{NS}
Panel 7	SW352	3	2.909	0.5827	0.583	0.656	1.084	0.111	24.000***
Panel 8	SW911	7	2.569	0.5799	0.235	0.611	1.326	0.615	74.970***
Panel 9	SO086	9	5.040	0.775	0.952	0.802	1.819	–0.188	26.648 ^{NS}
Mean overall loci		5.59±0.598	3.02±0.284	0.53±0.250	0.38±0.060	0.57±0.055	1.17±0.124	0.273	

* Significant ($P \leq 0.05$); **highly significant ($P \leq 0.01$); ^{NS} not significant ($P \geq 0.05$); 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.

Ghungroo pig 3.15±1.546 (Zaman *et al.* 2013a). 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) (Sollero *et al.* 2010). 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 to the major part of the allelic frequency at each locus. The PIC value in Votho pigs ranged from 0.0499 to 0.775 which corroborated with the mean PIC (0.49±0.171) in Meghalaya local pigs (Zaman *et al.* 2013b) and in Brazilian pig breeds mean PIC value of 0.655 (Sollero *et al.* 2010) reported earlier 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 Votho 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) (Sollero *et al.* 2010). The observed heterozygosity in Votho 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) (Swart *et al.* 2010). However, the heterozygosities observed in the investigation are not in accordance with the values reported for microsatellites in Indian pig populations (Kaul *et al.* 2001) and Chinese pig breeds (Li *et al.* 2000 a,b). The chi-square (χ^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. Shannon's information index (I), which measures the level of diversity, was sufficiently high with a mean of 1.17±0.124. The overall mean F_{IS} (0.273) observed in the present study indicating a 27.3% shortfall of heterozygosity in Votho pig population which may be

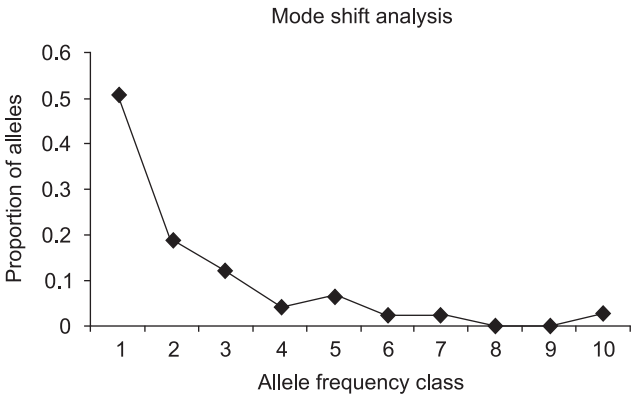

Fig. 1. Allele proportions and their contribution in Votho pig population.

Table 2. Bottleneck analysis in Votho pig

Model	Sign rank test - Number of loci with heterozygosity excess			Standardized differences test - T_2 values (probability)	Wilcoxon test - Probability of heterozygosity excess
	Expected	Observed	Probability		
IAM	12.17	7	0.01964	–2.560 (0.00523)	0.95560
TPM	12.24	7	0.01830	–5.080 (0.00000)	0.99645
SMM	12.46	5	0.00102	–8.449 (0.00000)	0.99970

IAM, Infinite allele model; TPM, two phase model; SMM, stepwise mutation model.

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.

Three mutation models namely, infinite allele model (IAM), two phase model (TPM), stepwise mutation model (SMM) were estimated using the programme Bottleneck (Table 2). The results indicated that Votho 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. 1).

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

SUMMARY

This study details genetic diversity of Votho pig population using a set of 21 microsatellite markers recommended by Food and Agriculture Organization of United Nations (FAO) for Swine. All the studied loci were highly informative. The number of observed alleles (N_a) detected ranged from 2 to 10, with an overall mean of 5.59±0.598. In total 122 alleles were observed across the investigated loci. The effective number of alleles (N_e) ranged from 1.054 to 5.075 with a mean of 3.02±0.284. The Polymorphic Information Content (PIC) value ranged from 0.0499 to 0.7750 with the overall mean of 0.53±0.250. The overall means for observed (H_o) and expected (H_e) heterozygosities were 0.38±0.060 and 0.57±0.055 respectively. The within breed inbreeding estimate indicated heterozygosity shortage of 0.273. The Hardy-Weinberg equilibrium (HWE) test revealed that 14 out of 21 loci deviated from equilibrium. Shannon's information index (I), was sufficiently high with a mean of 1.17±0.124. The bottleneck analysis revealed that population has not undergone any recent reduction.

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