

Microsatellite based diversity analysis of native pigs of North-Eastern India

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ABSTRACT

The native pigs of North Eastern region have unique traits such as early sexual maturity and quality bristles compared with exotic/ crossbreds. An attempt was made to measure the genetic variation available within Niang Megha and Tenyi Vo pigs using 22 FAO-ISAG microsatellite markers. The analysis of microsatellite data revealed average number of observed alleles as 6.95 ± 0.54 (Niang Megha) and 7.18 ± 0.45 (Tenyi Vo) where as the effective average number of alleles were 3.55 ± 0.33 and 3.81 ± 0.41 , respectively. The mean observed heterozygosities were found to be 0.61 ± 0.04 and 0.68 ± 0.05 , whereas expected values were 0.67 ± 0.03 and 0.69 ± 0.03 , for Niang Megha and Tenyi Vo pigs, respectively. Abundant genetic variations were displayed within the native pigs with the PIC values ranged between 0.41 (Sw2008) to 0.86. (S0218) for Niang Megha and between 0.45 (S0026) to 0.88 (S0005) in Tenyi Vo pigs. The mean F value was 0.15 ± 0.05 in Niang Megha which is more than that found in Tevyi Vo. There was no genetic bottleneck observed in the studied population. A total of 23 and 22 private alleles observed in Niang Megha and Tenyi Vo pigs.

Key words: Genetic variation, Microsatellite, Native pigs, Niang Megha, Population bottleneck, Tenyi Vo.

INTRODUCTION

Although, the exotic germplasm was introduced in India since early 1970s in systematic breeding programmes, most of the indigenous swine population (76% as per 2012 Census) is non-descriptive. Out of 10.29 million Indian pigs, more than half are housed in eastern and north eastern part of country which spreads over Indo-Burma global biodiversity hotspot. The comparative growth rates, feed conversion efficiency as well as multiplication rate are poor in indigenous pigs. However, they are unique due to adaptation to local soil, climate as well as system of rearing. They are also evaluated in the zero input system since time immemorial to produce high quality pork from byproducts and garbage. They bear unique features such as better heat tolerance, meat quality, early sexual maturity (Karunakaran *et al.*, 2009; Kumaresan *et al.*, 2008) and good quality bristles (Mohana *et al.*, 2014) compared with exotic/ crossbreds. They have been maintained in scavenging system and act mainly as insurance to the rural livelihood in rain fed agriculture. While there is paucity of literature on characterization of these lesser known strain/breeds, the economic and social backwardness of the majority stakeholders further delays the process. The phenotypic as well as genetic characterization has taken pace these days in India for cataloguing these distinct populations. So far three native pigs have been characterized and accorded status of indigenous registered breeds which was initiated for the first time only in 2012 (NBAGR, 2015) and in this process had been catalogued and some more are in pipeline. Furthermore,

while a number of factors potentially contribute to the conservation decision making, mostly a primary objective of a conservation programme is to preserve as much genetic diversity as possible (Boettcher *et al.*, 2010). Therefore assessment of the genetic variation of indigenous pigs is an essential task for animal genetics studies as well as conservation decision making. Niang Megha is a unique germplasm of Meghalaya state producing pork, bristle and attached with the socio-cultural life of people of the state. They are black coloured medium sized pigs with thickly covered with high quality bristles at dorsal midline. They have ventral line almost touches the ground with 5-7 pairs of needle type in the mid ventral region. They have small erect ears extend in vertical direction and smaller and stronger forelegs with partial hoof placement. The Tenyi Vo (Yet to be considered as a breed) is a small sized early maturing (Kumarresan *et al.*, 2008, Karunakaran *et al.*, 2009) black colored pig found in the state of Nagaland. This is a small sized pot bellied type pig with a long straight snout and short erect ears. The present study aims to characterize these native pigs of north east India using the FAO-ISAG microsatellite markers in order to find out the genetic variation that exists within the breed to facilitate decision making on genetic cataloguing and conservation.

MATERIALS AND METHODS

Animals, sampling and microsatellite loci: The study included a total of 87 pigs (46 Niang Megha and 43 Tenyi Vo) which were selected from their respective breeding tract

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(Fig1). The breeding tract of Niang Megha pig covers mostly the Khasi, Garo, Jaintia mountain ranges of Meghalaya. This pig is found in almost all parts of the state but the areas which are inaccessible have mostly the purebred indigenous population. The breeding tract extends from Longitude: 89.46°E to 94.36°E and Latitude: 25.05°N to 26.41°N which consists of 7 districts viz. East Garo Hills, East Khasi Hills, Jaintia Hills, Ri-Bhoi, South Garo Hills, West Garo Hills and West Khasi Hills. The breeding tract of Tenyi Vo pig is mostly mountainous except those areas bordering Assam valley. It is also found near Mount Saramati which forms a natural barrier between Nagaland and Burma. It lies between the parallels of 98°E to 96°E longitude and 26.6°N to 27.4°N latitude north of the equator. The evergreen tropical and the sub tropical forests are found in strategic pockets in this tract. The native pigs, which looked alike and lacked the history of cross breeding (as per the local people) were selected from their breeding tracts and evaluated for their phenotypic breed characteristics as per the designed breed descriptor (Anonymous 2008). To ensure un-relatedness only two pigs

from each village were sampled for the study. The International Society of Animal Genetics (ISAG) recommended panel of 30 microsatellite loci for the genetic diversity evaluation of global pig breeds was attempted out of which 22 pairs of primers (S0026, S0155, S0005, Sw2410, Sw830, S0355, Sw24, Sw632, Swr1941, Sw936, S0218, Sw122, S0097, IGF1, Sw2406, Sw72, S0226, Sw2008, S0101, S0143, S0068, S0178) gave good amplification. All animals were genotyped for those 22 fluorescence-labeled microsatellite markers amplified in four multiplex PCRs (Table 1).

Blood collection, DNA extraction and microsatellite profiling: Blood samples were collected from the pigs of aseptically into vacutainers (Becton and Dickinson, USA) with EDTA as anticoagulant from the anterior vena cava by holding the animal in dorsal recumbency. Genomic DNA was isolated from the leucocytes of blood samples by the method as described by Sambrook and Russell (2001) using Proteinase-K and Phenol. The isolated genomic DNA was

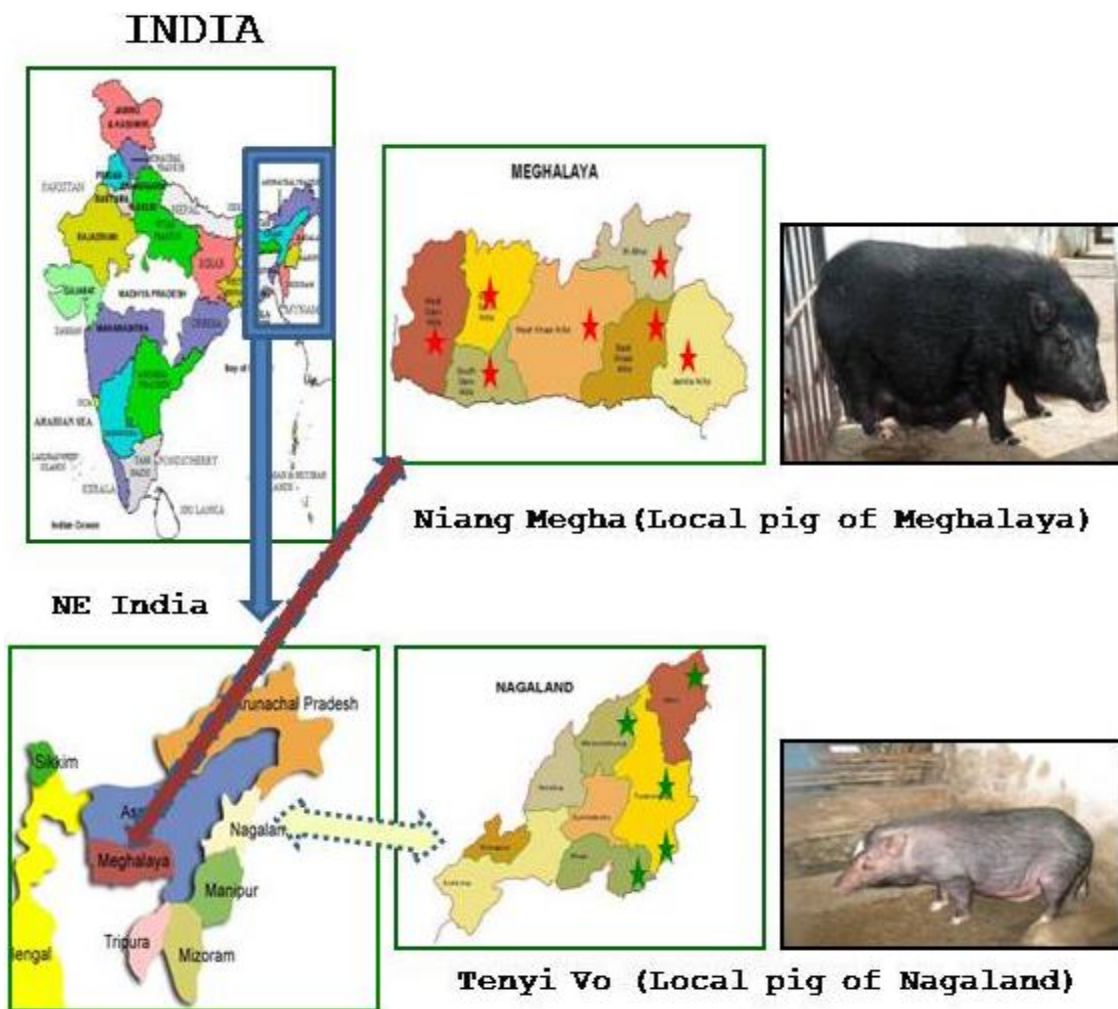


Fig 1: Breeding tracts of native pigs used in study

TABLE 1: FAO-ISAG panel of Microsatellite loci, their primer sequences and PCR conditions.

Locus	Primer sequence (5' -> 3') D- indicates Dye labelled primer	Annealing temp (°C)	MgCl ₂ conc. (μM)	Florescent Dye	Multiplex
S0026	D-AACCTTCCCTTCCCAATCAC CACAGACTGCTTTTACTCC	58	1.8	FAM	M ₂
S0155	D-TGTTCTCTGTTTCTCCTCTGTTTG AAAGTGGAAGAGTCAATGGCTAT	58	1.8	VIC	M ₂
S0005	D-TCCTTCCCTCCTGGTAACTA GCACTTCCTGATTCTGGGTA	60	1.8	FAM	M ₃
Sw2410	D-ATTTGCCCCCAAGGTATTTT CAGGGTGTGGAGGGTAGAAG	56	1.5	FAM	M ₁
Sw830	D-AAGTACCATGGAGAGGGAAATG ACATGGTTCCAAAGACCTGTG	56	1.5	VIC	M ₁
S0355	D-TCTGGCTCCTACACTCCTTCTTGATG TTGGGTGGGTGCTGAAAAATAGGA	56	1.5	PET	M ₁
Sw24	D-CTTTGGGTGGAGTGTGTGC ATCCAAATGCTGCAAGCG	60	1.8	PET	M ₃
Sw632	D-TGGGTTGAAAGATTTCCCAA GGAGTCAGTACTTTGGCTTGA	60	1.8	VIC	M ₃
Swr1941	D-AGAAAGCAATTTGATTTGCATAATC ACAAGGACCTACTGTATAGCACAGG	62	2.0	NED	M ₄
Sw936	D-TCTGGAGCTAGCATAAGTGCC GTGCAAGTACACATGCAGGG	58	1.8	NED	M ₂
S0218	D-GTGTAGGCTGGCGGTTGT CCCTGAAACCTAAAGCAAAG	58	1.8	PET	M ₂
Sw122	D-CAAAAAAGGCAAAAGATTGACA TTGTCTTTTTATTTTGCTTTTGG	56	1.5	HEX	M ₁
S0097	D-GACCTATCTAATGTCATTATAGT TTCCTCCTAGAGTTGACAACTT	56	1.5	HEX	M ₁
IGF1	D-GCTTGGATGGACCATGTTG CATATTTTCTGCATAACTTGAACCT	56	1.5	HEX	M ₁
Sw2406	D-AATGTCACCTTTAAGACGTGGG AATGCGAAACTCCTGAATTAGC	58	1.8	HEX	M ₂
Sw72	D-ATCAGAACAGTGCGCCGT TTTGAAAATGGGGTGTTC	56	1.5	FAM	M ₁
S0226	D-GCACTTTTAACTTTCATGATACTCC GGTTAAACTTTNCCCCAATACA	58	1.8	FAM	M ₂
Sw2008	D-CAGGCCAGAGTAGCGTGC CAGTCCTCCCAAAAATAACATG	58	1.8	HEX	M ₂
S0101	D-GAATGCAAAGAGTTCAGTGTAGG GTCTCCCTCACACTTACCGCAG	60	1.8	FAM	M ₃
S0143	D-ACTCACAGCTTGTCTGGGTGT CAGTCAGCAGGCTGACAAAAC	62	2.0	FAM	M ₄
S0068	D-CCTTCAACCTTTGAGCAAGAAC AGTGGTCTCTCCCTCTTGCT	62	2.0	FAM	M ₄
S0178	D-TAGCCTGGGAACCTCCACACGCTG GGCACCAGGAATCTGCAATCCAGT	60	1.8	HEX	M ₃

checked for quality, purity and concentration. Only the DNA samples of good quality, purity and concentration of $\geq 100\text{ng}/\mu\text{L}$ were used for further analysis. All animals were genotyped for 22 fluorescence-labeled microsatellite markers amplified in four multiplex PCRs. The multiplex-PCRs were performed in a total of 25 μL volume containing 50 to 100 ng of porcine genomic DNA as template, 1 X PCR buffer, 0.1 to 1.0 mM of forward (fluorescence labeled with FAM, VIC, PET, NED and HEX) and reverse primers, 200 mM of each dNTP,

2.5mM MgCl₂ and 1 U of Taq polymerase (FERMENTAS). The reactions were performed on the ABI thermal cycler (Veriti, Applied Biosystem, USA) under the thermal cycle profile: denaturation at 95°C for 10 minutes in the first cycle, 35 cycles of 95°C for 45 seconds, 56–62 °C for 45 seconds, 72°C for 45 seconds, and extension at 72 °C for 10 minutes for the last cycle. Electrophoresis in 1% agarose gel was used to make sure that PCR products were well amplified. The multiplex-PCR products were genotyped using capillary

electrophoresis with fluorescent detection (ABI 3730 DNA Analyzer, Applied Biosystems, USA). The PCR products were diluted with deionized water and then mixed with Hi-Di formaldehyde (Applied Biosystems, USA) and the internal size standard (GeneScan-500 LIZ Size Standard, Applied Biosystems, USA). The mixtures were loaded into a 96-well plate for detection of the fragment size of PCR products. The fragment size was calibrated with Peak Scanner Software version 1.0 (ABI PRISM, Applied Biosystems, USA). Representative amplified products were cloned and sequenced to confirm the result and the amplified fragments for which no accession was available were submitted to Gen bank (JQ342092; JQ396262; JQ396261).

Statistical analysis: To study the genetic variation analysis/ levels of variation at each locus, different measurements of within breed genetic variations, viz. observed number of alleles (Na), effective number of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He) were estimated using POPGENE software package (Yeh *et al.*, 1999). The polymorphism information content (PIC) was calculated by the formula given by Botstein *et al.* (1980) with the EXCEL Toolkit. Allele frequencies at each locus, the average number of observed and effective alleles of the 22 microsatellite markers and observed and expected heterozygosities were also computed. Population differentiation was assessed using the microsatellite-based measure of differentiation-FST calculated with the program FSTAT (Goudet, 1995). Allele frequency distribution of the microsatellite loci was examined by using program Bottleneck 1.2.02, for mode shift (Luikart *et al.*, 1998a,b), which may indicate if a recent genetic bottleneck has occurred. Three models namely Infinite Allele Model (I.A.M); Two-phased model of mutation (T.P.M) and Stepwise Mutation Model (S.M.M) were employed and three statistical tests are performed for each mutation model the allele frequency distribution is established in order to see whether it is a mode shift. To determine whether a population exhibits a significant number of loci with gene diversity excess, three tests, namely a “sign test”, a “standardized differences test” and a “Wilcoxon sign-rank test” were employed.

RESULTS AND DISCUSSION

The approach for conservation should be a subset of total diversity logically determined using suitable decision support system (may be with molecular genetic information) to reduce the pressure on taxpayers' pockets. Therefore, the genetic resources which are yet to be evaluated and characterized are getting priority to explore their probable conservation strategy. In India the characterization and cataloguing was delayed to a great extent and only in 2012 two of the native breeds were catalogued. The breeding tracts of these two pigs (Fig.1) are geographically separated by several mountains, rivers and hilly terrains. Therefore the

availability of sufficient genetic variation needs to be ensured before going for any genetic improvement and conservation programme.

Genetic variability of microsatellite loci: A total of 153 and 158 alleles were observed in the 22 microsatellites in Niang Megha and Tenyi Vo respectively; polymorphisms at all loci were observed in the examined populations. The allele size range, observed and effective number of alleles observed and expected heterozygosity and PIC at 22 studied Microsatellite loci in native Indian pigs are given in Table 2. The allele size range varied from 204-216 bp at locus Swr1942 to 203-269 at locus S0005 in case of Niang Megha whereas 95-102 (Sw2008) to 203-261 (S0005) for Tenyi Vo. The total observed number of alleles ranged between 4 (S0097) and 12 (S0005 and S0178) for Niang Megha whereas from 4 (Sw2008) to 12 (Sw936) for Tenyi Vo. The genetic diversity within the population is assessed by effective number of alleles and heterozygosity. The effective number of alleles ranged from 1.70 (Sw2008) to 6.76 (S0005) for Niang Megha whereas from 1.87 (S0101) to 8.53 (S0005) in Tenyi Vo with mean of 3.55 ± 0.54 and 3.81 ± 0.41 , respectively. The mean observed heterozygosities are lower than the expected values based on these 22 studied loci. The observed and expected heterozygosities ranged from 0.08 to 0.89 and 0.41 to 0.86 in Niang Megha whereas from 0.13 to 0.90 and 0.46 to 0.88 in Tenyi Vo pigs. The genetic diversity of LWY pigs or other European pig breeds were also reported in various other studies (Fredholm *et al.*, 1993; van Zeveran *et al.*, 1995; Laval *et al.*, 2000; Martinez *et al.*, 2000). However, the high genetic diversity have been reported earlier in Desi and Gahuri pigs by us (Behl *et al.*, 2002 and Behl *et al.*, 2006) and also in Chinese and Mexican pig populations (Lemus Flores *et al.*, 2001; Fang *et al.*, 2005). A total of 23 and 22 private alleles were observed for Niang Megha and Tenyi Vo pigs, respectively.

Polymorphic information content (PIC): The PIC is a parameter indicative of the informative degree of a marker. PIC for all the 22 markers is shown in Table 2. The PIC value can range from 0 to 1, however, range of PIC estimates in this study were ranging from 0.41 (Sw2008) to 0.86 (S0218) for Niang Megha whereas from 0.45 (S0026) to 0.88 (S0005) in Tenyi Vo with mean of 0.65 ± 0.03 and 0.68 ± 0.03 , respectively. Similar results were also reported in other pigs (Laval *et al.*, 2000; Martinez *et al.*, 2000; Behl *et al.*, 2002; Li *et al.*, 2010; Wang *et al.*, 2004). Most of the markers had PIC values higher than 0.5, which is a useful indicator of genetic variability and forms the basis for developing breeding or genetic improvement strategy for a population. The present study resulted in identification of five highly polymorphic SSR loci viz., S0005, Sw 936, S 0218, S0226 and S0178 for Niang Megha and S0005, Sw 24, Sw 936, Sw122 and S0178 for Tenyi Vo respectively. These polymorphic primers can effectively be used in further

Table 2: The population genetic variation at 22 FAO-ISAG panels of microsatellite loci in North East Indian pigs.

Locus	Niang Megha (Local pig of Meghalaya)							Tenyi Vo (Local pig of Nagaland)						
	Na	Ne	Ho	He	Allelic range	PIC	F	Na	Ne	Ho	He	Allelic range	PIC	F
S0026	6	2.30	0.36	0.57	87-103	0.52	0.36	6	1.89	0.50	0.47	85-97	0.45	-0.06
S0155	6	2.75	0.90	0.64	142-62	0.64	0.41	6	3.03	0.90	0.67	142-62	0.66	0.34
S0005	12	6.76	0.61	0.85	203-69	0.85	0.29	11	8.53	0.78	0.88	203-61	0.88	0.12
Sw2410	5	3.48	0.50	0.71	106-26	0.67	0.30	6	4.05	0.94	0.75	104-22	0.75	-0.25
Sw830	9	2.46	0.50	0.59	172-203	0.56	0.16	6	3.12	0.78	0.68	168-203	0.67	-0.15
S0355	6	2.41	0.63	0.58	248-70	0.53	-0.08	8	4.13	0.69	0.76	244-70	0.75	0.08
Sw24	4	2.48	0.71	0.59	95-119	0.55	-0.18	7	4.52	0.90	0.78	95-119	0.78	-0.16
Sw632	5	2.64	0.64	0.62	154-70	0.59	-0.03	6	2.36	0.44	0.58	148-68	0.54	0.25
Sw1941	4	2.73	0.82	0.63	204-16	0.63	-0.29	6	2.72	0.78	0.63	202-16	0.63	-0.24
Sw936	10	5.83	0.79	0.83	92-112	0.82	0.05	12	7.95	0.96	0.87	90-112	0.87	-0.10
S0218	11	7.33	0.71	0.86	160-201	0.86	0.17	10	3.18	0.59	0.69	158-88	0.66	0.14
Sw122	5	2.61	0.61	0.62	106-22	0.57	0.02	9	6.74	0.96	0.85	106-22	0.85	-0.12
S0097	4	2.07	0.29	0.52	213-34	0.49	0.45	5	2.16	0.70	0.54	213-34	0.54	-0.29
IGF1	6	3.70	0.5	0.73	195-207	0.72	0.32	6	4.60	0.74	0.78	195-207	0.78	0.06
Sw2406	9	2.94	0.64	0.66	224-54	0.65	0.03	10	5.13	0.86	0.81	222-56	0.80	-0.07
Sw72	6	2.72	0.58	0.63	99-113	0.63	0.09	5	2.98	0.84	0.67	99-113	0.66	-0.27
S0226	7	4.74	0.68	0.79	182-208	0.77	0.14	5	2.025	0.17	0.51	190-98	0.47	0.67
Sw2008	6	1.70	0.08	0.41	95-108	0.41	0.81	4	2.48	0.13	0.60	95-102	0.55	0.78
S0101	8	4.75	0.64	0.79	197-221	0.77	0.19	6	1.87	0.38	0.46	197-215	0.46	0.19
S0143	7	3.61	0.89	0.72	152-65	0.72	-0.23	8	2.78	0.57	0.64	150-67	0.64	0.12
S0068	5	2.82	0.45	0.65	225-36	0.60	0.30	8	4.18	0.62	0.76	213-40	0.75	0.19
S0178	12	5.35	0.82	0.81	105-28	0.82	-0.01	8	4.25	0.83	0.77	107-24	0.76	-0.08
Mean	6.95±0.54	3.55±0.33	0.61±0.04	0.67±0.03	-	0.65±0.03	0.15±0.05	7.18±0.45	3.81±0.41	0.68±0.05	0.69±0.03	-	0.68±0.03	0.05±0.06

Na: observed number of alleles, Ne: effective number of alleles, Ho: observed heterozygosity, He: expected heterozygosity, PIC: Polymorphism information content, F: Fixation Index

molecular breeding programs since they exhibited very high polymorphism over other loci. SSR analysis resulted in a more definitive separation of clustering of genotypes indicating a higher level of efficiency of SSR markers for the accurate determination of relationships.

F estimates: The inbreeding coefficient measures the reduction of heterozygosity because of nonrandom mating within the population. Hence F value greater or lesser than zero reveals inbreeding or out breeding. Inbreeding coefficients for all markers used in this study are given in Table 2. Six loci in case of Niang Megha and 11 loci in Tenyi Vo revealed negative F value ($F < 0$) indicating absence of inbreeding at these loci. Average F value for markers ranged from 0.002 (Sw122) to 0.81 (Sw2008) in Niang Megha whereas from 0.08 (S0355) to 0.78 (Sw2008) in Tenyi Vo with mean of 0.15 ± 0.05 and 0.05 ± 0.06 , respectively. Among the negative values it ranged from -0.29 (Swr1941) to -0.01 (S0178) in Niang Megha whereas from -0.06 (S0026) to -0.29 (S0097) in Tenyi Vo, respectively. The mean lower F value indicated the nominal amount of inbreeding in these populations. The higher negative F indicated presence of heterozygosity suggesting that these populations might have been managed under controlled mating system by avoiding mating between the close relatives. Similar values were also reported by other studies. (Li *et al.*, 2010; Li *et al.*, 2015; Wang *et al.*, 2004 and Wang *et al.*, 2011)

Genetic bottleneck analysis: In a recently bottlenecked population, the observed gene diversity is higher than the expected equilibrium gene diversity because the allele numbers is reduced faster than the gene diversity which can be computed from the N_e , under the assumption of a constant-size (equilibrium) population (Luikart *et al.*, 1998a). In a population at mutation-drift equilibrium, there is approximately an equal probability that a locus shows gene diversity excess or a gene diversity deficit. Three models were employed and three statistical tests are performed for each mutation model the allele frequency distribution is established in order to see whether it is approximately L-shaped (as expected under mutation-drift equilibrium) or not (recent bottlenecks provoke a mode shift). The results of the bottle neck analysis of native pigs using three tests (I.A.M., T.P.M and S.M.M) have been presented in Table 3.

In these native pigs, under Sign test, the expected numbers of loci with heterozygosity excess were 13.0 (IAM), 13.1 (TPM) and 13.0 (SMM) for Niang Megha and almost similar in case of Tenyi Vo. The observed values were higher, equal and lower than the observed numbers of loci. As the previous reports explained most microsatellite data sets better fit the TPM (Two-phased model of mutation) than the SMM (Stepwise Mutation Model) or IAM (Infinite Allele Model). Here in case of heterozygosity excess under TPM was not significantly ($P > 0.05$) lower than the observed numbers of loci, the null hypothesis that the population is under mutation-drift equilibrium was accepted. Also the mode shift indicator i.e. qualitative method of estimation of bottleneck, for a mode shift in allele frequency classes with 22 microsatellite loci as per earlier recommendations of 8-10 loci (Spencer *et al.*, 2000; Arora and Bhatia, 2009) showed the normal L-shaped curve (Fig. 2) in graphical representation of proportion of alleles verses class of frequency distribution. The L shaped curve indicated the abundance of low frequency (< 0.10) alleles. This finding suggested the absence of any detectably large, recent genetic bottleneck (last 40-80 generations) in declining population, where the probability of low frequency allele's loss was very high. Under Wilcoxon rank test, probability values were 0.05, 0.83 and 0.99 in Niang Megha and 0.00, 0.16 and 0.99 in Tenyi Vo for IAM, TPM and SMM; these were significant ($P < 0.05$) in case of IAM only. In conclusion, the panel of microsatellites evaluated native pigs of North East India in the present study showed high

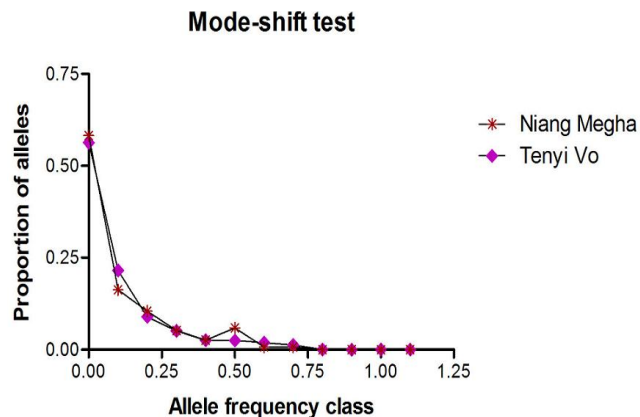


Fig 2: Mode-shift test for bottleneck in native Indian pigs

Table 3: Population bottle neck analysis in native Indian pigs

Model used in the study		Niang Megha			Tenyi Vo		
		I.A.M.	T.P.M.	S.M.M.	I.A.M.	T.P.M.	S.M.M.
Sign test (No. of loci with heterozygosity excess)	Exp	13.0	13.1	13.0	13.1	13.2	13.0
	Obs	16.0	12.0	4.0	22.0	13.0	5.0
	P- value	0.1	0.4	0.1×10^{-3}	0.1×10^{-4}	0.5	0.5×10^{-3}
Standardized differences test	T2 values	1.0	-2.0	-7.4	3.4	0.8	-3.9
	P- value	0.1	0.02	0.00	0.4×10^{-3}	0.2	0.5×10^{-4}
Wilcoxon test (one tail for H excess)	P- value	0.05	0.83	0.99	0.00	0.16	0.99

I.A.M.: Infinite Allele Model, T.P.M.: Two-phased model of mutation, S.M.M.: Stepwise Mutation Model

heterozygosity and polymorphism indicating abundance of genetic variation stored in this pig breed. However study demonstrates that although genetic variability was not reduced significantly in native Indian pigs, there are indications that some variability has been lost shown by certain degree of heterozygosity deficiency. The present study clearly verified that using these panel of microsatellites markers different breeds or populations of native Indian pigs can be suitably investigated to help conservation planning

in this regard as fewer efforts will be required to conserve a genetically healthy population rather than a genetically impoverished one.

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REFERENCES

- Anonymous (2008) Annual Report (2007-08). ICAR-National Research Centre on Pigs. Rani, Guwahati. Pp: 1-42
- Arora, R. and Bhatia, S. (2009). Evaluation of genetic effects of demographic bottleneck in Muzzafarnagri Sheep from India using microsatellite markers. *Asian-Aust. J. Anim. Sci.*, **22**:1-6.
- Behl, R., Kaul R., Sheoran N., Behl J., Tania M.S. and Vijh R.K. (2002). Genetic identity of two Indian pig types using microsatellite markers. *Anim. Genet.*, **33**: 158-159.
- Behl, R., Sheoran N., Behl J. and Vijh R.K. (2006). Genetic analysis of Ankamali pigs of India using microsatellite markers and their comparison with other domesticated Indian pig types. *J. Anim. Breed. Genet.*, **123**: 131-5.
- Boettcher, P.J., Tixier-Boichard M., Toro M., Simianer H., Eding H., Gandini G., Joost S., Garcin D., Colli L., Ajmone-Marsan P. and GLOBALDIV Consortium. (2010). Objectives, criteria and methods for using molecular genetic data in priority setting for conservation of animal genetic resources. *Anim. Genet.*, **41**: 64-77.
- Botstein, D., White R.L., Skolnick M. and Davis R.W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.*, **32**: 314-331.
- Fang, M., Hu X., Jiang T., Braunsweig M., Hu L., Du Z., Feng J., Zhang Q., Wu C. and Li N. (2005). The phylogeny of Chinese indigenous pig breeds inferred from microsatellite markers. *Anim. Genet.*, **36**: 7-13.
- Fredholm, M., Wintero A.K., Christensen K., Kristensen B., Nielsen P.B., Davies W. and Archibald A. (1993). Characterization of 24 porcine (AC)n(TG)n microsatellites: Genotyping of unrelated animals from four breeds and linkage studies. *Mammalian Genome*, **4**:187-192.
- Goudet, J. (1995). FSTAT (vers. 2.9.3): a computer program to calculate F-statistics. *J. Hered.*, **86**:485-486.
- Karunakaran, M., Mandal M., Rajarajan K., Karmakar H.D., Bhatt B.P., Das J., Bora B., Baruah K.K. and Rajkhowa C. (2009). Early puberty in local Naga boar of India: Assessment through epididymal spermogram and in vivo pregnancy. *Anim. Reprod. Sci.*, **111**: 112-119.
- Kumaresan, A., Bujarbaruah K.M., Karunakaran M., Das A. and Bardoloi R.K. (2008). Assessment of early sexual maturity in nondescript local pigs of north east India: testicular development, spermogram and in vivo pregnancy. *Livestock Sci.*, **116**: 342-347.
- Laval, G., Iannuccelli N. and Legault C., *et al.* (2000). Genetic diversity of eleven European pig breeds. *Genet. Sel. Evol.*, **32**:187-203.
- Lemus Flores, C., Ulloa-Arvizu R., Ramos-Kuri M., Estrada F. J. and Alonso R. A. (2001). Genetic analysis of Mexican hairless pig populations. *J. Anim. Sci.*, **79**: 3021-3026.
- Li, K.Y., Li K.T., Cheng C.C., Chen C.H., Hung C.Y., Ju Y.T. (2015). A genetic analysis of Taoyuan pig and its phylogenetic relationship to Eurasian pig breeds. *Asian-Aust. J. Anim. Sci.*, **28**: 457-466.
- Li, X., Li K., Fan B., Gong Y., Zhao S., Peng Z. and Liu B. (2000). The genetic diversity of seven pig breeds in China, estimated by means of microsatellites. *Asian-Aust. J. Anim. Sci.*, **13**: 1193-1195.
- Luikart, G., Allendorf F.W., Cornuet J.M. and Sherwin W.B. (1998a). Distortion of allele frequency distributions provides a test for recent population bottleneck. *J. Hered.*, **89**: 238-247.
- Luikart G., Sherwin W.B., Steele B.M. and Allendorf F.W. (1998b). Usefulness of molecular markers for detecting population bottlenecks via monitoring genetic change. *Mol. Ecolo.*, **7**: 963-974.
- Martinez, A.M., Delgado J.V., Rodero A. and Vega-Pla J.L. (2000). Genetic structure of Iberian pig breeds using microsatellites. *Anim. Genet.*, **31**: 295-301.
- Mohana, N.H., Debnath S., Mahapatra R.K., Nayak L.K., Baruah S., Das A., Banik S. and Tamuli M. K. (2014). Tensile properties of hair fibres obtained from different breeds of pigs. *Biosyst. Engineer.*, **119**: 35-43

- National Bureau of Animal Genetic Resources. (2015). <http://www.nbagr.res.in/registeredbreed.html>
- Sahoo, N.R. Das A, Naskar S., Banik S. and Tamuli M.K. (2012) *Niang Megha: The nature's gift for food and fibre*. National Research Centre on Pig (ICAR), Rani, Guwahati. Pp: 1-30
- Sambrook J. and Russell D.W. (2001). *Molecular Cloning, a Laboratory Manual*. 3rd Edition, Cold Spring Harbor Laboratory Press, NY, USA.
- 19th livestock Census. (2012). Department of Animal Husbandry, Dairy & Fisheries, M/ O Agriculture. Govt of India.
- Spencer, C.C., Neigel J.E. and Leberg P.L. (2000). Experimental evaluation of the usefulness of microsatellite DNA for detecting bottlenecks. *Mol. Ecolo.*, **9**: 1517-1528.
- Van-Zeveran, A., Peelman L., Van de Weghe A. and Bouquet Y. (1995). A genetic study of four Belgian pig populations by means of seven microsatellite loci. *J. Anim. Breed. Genet.*, **112**: 191–204.
- Wang, X., Cao H. H., Geng S. M. and Li H. B. (2004). Genetic Diversity of 10 Indigenous Pig Breeds in China by Using Microsatellite Markers. *Asian-Aust. J. Anim. Sci.*, **17**: 1219-1222.
- Wang, J.Y., Guo J. F., Zhang Q., Hu H. M., Lin H. C., Wang C., Zhang Y. and Wu Y. (2011). Genetic diversity of Chinese Indigenous pig breeds in Shandong Province using microsatellite markers. *Asian-Aust. J. Anim. Sci.*, **24**: 28-36.
- Yeh, F.C., Yang R. and Boyle T. (1999). POPGENE Version 1.31, Microsoft Window Based Freeware for Population Genetic Analysis. University of Alberta and Centre for International Forestry Research.