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Genetic diversity analysis of Ghoongroo pig based on microsatellite markers

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ABSTRACT

An attempt was made to measure the genetic variation available within Ghoongroo pigs, a prized germplasm in terms of high growth and multiplication rate among the indigenous varieties, using 22 FAO-ISAG microsatellite markers. The data were analyzed applying different software to estimate the various measures of genetic diversity. The average number of observed allele was 8.18±0.62 and the effective average number of allele was 4.39±0.26. The observed heterozygosity ranged from 0.4 to 0.96 and the expected heterozygosity from 0.55 to 0.85. Average PIC value ranged from 0.55 (S0026) to 0.85 (S0068) with the average value 0.75±0.02. The mean F value was 0.07±0.04. There was no genetic bottleneck observed in the studied population. A total of 45 private alleles were observed which can be used as breed specific markers. The results suggested that all the microsatellite markers were highly polymorphic and suitable for molecular characterization of Ghoongroo pig. There was substantial genetic variation and polymorphism across the studied loci. The low inbreeding observed was a favourable parameter to formulate the appropriate breeding strategies to enhance heterozygosity in the population.

Key words: Genetic variation, Ghoongroo pig, Microsatellite markers, Population bottleneck

Several factors potentially contribute to the decision making on the priority of breeds/strains for conservation but mostly the primary objective of a conservation programme is to preserve as much genetic diversity as possible (Boettcher *et al.* 2010). There is paucity of literature on characterization of lesser known strain/breeds of indigenous pigs and the economic and social backwardness of the majority stakeholders further delays the process. Although the growth rate and feed conversion efficiency of native Indian pigs are lower, yet they have unique features such as better heat tolerance, meat quality, early sexual maturity (Karunakaran *et al.* 2009, Kumaresan *et al.* 2008) and quality bristles (Mohana *et al.* 2014) compared with exotic/ crossbreds. They are evaluated under the zero-input system since time immemorial for producing quality pork from byproducts. Although, exotic germplasm was introduced in India in 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 pigs, more than half are found in eastern and north eastern part of the country. Ghoongroo, a domesticated native pig of Eastern India is a prized germplasm in terms of high growth and multiplication rate among the indigenous varieties. This is a medium size, black color pig with proportionately larger body size with a longer barrel than other local strains found in Eastern India (Sahoo *et al.* 2013). These are docile and easy to handle and is well adapted with the climate, soil and system of rearing. The present investigation aims to characterize the native Indian registered Ghoongroo pig using the FAO-ISAG microsatellite markers to facilitate decision making on cataloguing, genetic improvement as well as conservation priority.

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MATERIALS AND METHODS

Animals, sampling and microsatellite loci: Ghoongroo pigs (45), which were selected from the breeding tract (Fig.1) were used. The native pigs, which looked alike and lacked the history of crossbreeding were selected from their breeding tracts and evaluated for their phenotypic breed characteristics as per the breed descriptor. To ensure un-relatedness only 2 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

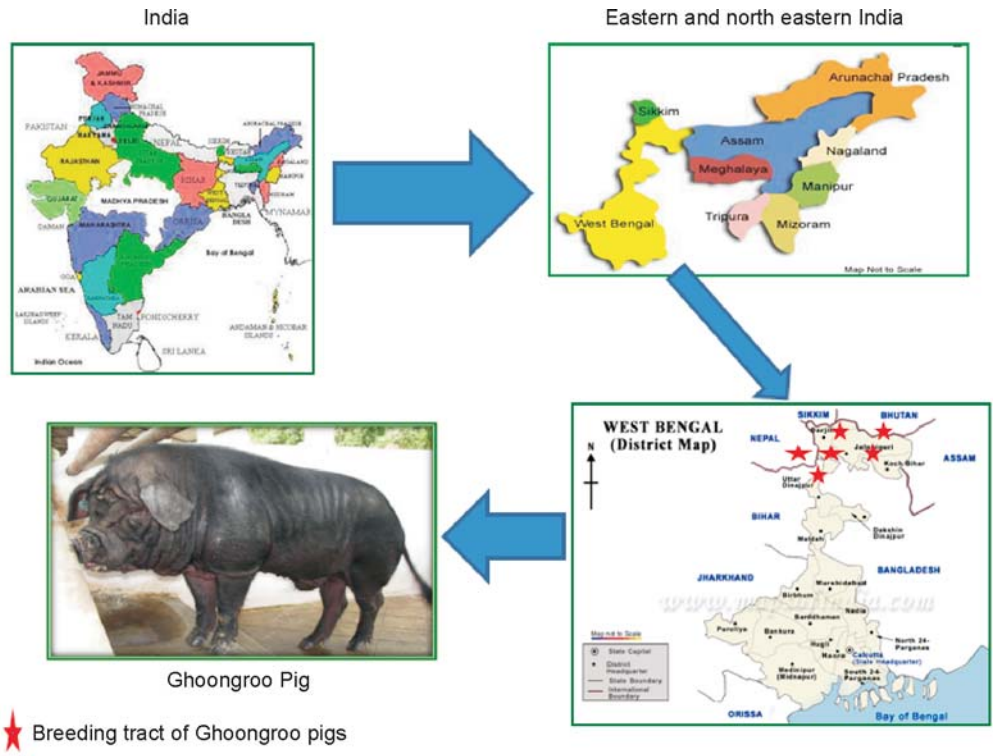

Fig. 1. Breeding tract of Ghoongroo pig used in study.

in four multiplex PCRs.

Blood collection, DNA extraction and microsatellite profiling: Blood samples were collected aseptically from the pigs into vacutainers containing 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 (Sambrook and Russell 2001) using proteinase-K and phenol. The genomic DNA isolated was checked for quality, purity and concentration. Only the DNA samples of good quality, purity and concentration were used for further analysis. All animals were genotyped for those 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 $MgCl_2$ and 1 U of Taq polymerase. The reactions were performed on the thermal cycler under the thermal cycle profile: denaturation at 95º C for 10 min in the first cycle, 35 cycles of 95º C for 45 sec, 56–62 °C for 45 sec, 72 ºC for 45 sec, and extension at 72 ºC for 10 min 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. The PCR products were diluted with deionized water and then mixed with formaldehyde and the internal size standard. 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. 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 (JQ342091).

Statistical analysis: For 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. 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. To determine whether a population exhibits a significant number of loci with gene diversity excess, 3 tests, viz. sign test, standardized differences test and Wilcoxon sign-rank test were used.

RESULTS AND DISCUSSION

A wide agreement exists on the need to conserve genetic diversity of AnGR globally but at the same time all of them cannot be conserved, hence a process and/or need to prioritize animal genetic resources for conservation are necessary (Boettcher *et al.* 2010). The approach should be conservation of a subset of total diversity logically

Table 1. The population genetic variability in Ghoongroo pigs at ISAG microsatellite loci

ISAG Locus	Na	Ne	Ho	He	Allelic range	PIC	F
S0026	5	2.2	0.74	0.55	89–103	0.55	–0.35
S0155	6	3.57	0.93	0.72	142–62	0.72	0.29
S0005	13	6.65	0.76	0.85	245–69	0.84	0.10
Sw2410	10	4.32	0.77	0.77	92–130	0.76	0.002
Sw830	5	4.31	0.8	0.77	180–91	0.75	–0.02
S0355	10	5.20	0.78	0.81	244–70	0.79	0.03
Sw24	10	5.46	0.96	0.82	93–124	0.82	–0.18
Sw632	8	3.83	0.67	0.74	148–72	0.74	0.099
Swr1941	6	3.38	0.54	0.70	202–20	0.70	0.23
Sw936	10	4.87	0.86	0.79	90–112	0.79	–0.08
S0218	7	3.01	0.6	0.67	158–82	0.67	0.10
Sw122	9	4.45	0.85	0.78	106–22	0.77	–0.10
S0097	6	3.53	0.6	0.72	213–40	0.71	0.16
IGF1	7	3.71	0.58	0.73	193–207	0.72	0.21
Sw2406	11	4.65	0.47	0.79	222–48	0.78	0. 40
Sw72	6	5.19	0.73	0.81	99–113	0. 8	0.099
S0226	10	5.66	0.5	0.82	180–210	0.82	0.39
Sw2008	3	2.35	0.46	0.58	95–102	0.57	0.21
S0101	5	3.36	0.4	0.70	203–15	0.70	0.43
S0143	8	5.40	0.97	0.82	152–67	0.81	–0.19
S0068	15	6.75	0.72	0.85	211–46	0.85	0.15
S0178	10	4.75	0.89	0.79	101–26	0.79	–0.13
Mean±SE	8.18 ±0.62	4.39 ±0.26	0.71 ±0.04	0.75 ±0.02	-	0.75 ±0.02	0.07 ±0.04

Na, observed number of alleles; Ne, effective number of alleles; Ho, observed heterozygosity; He, expected heterozygosity. PIC, polymorphism information content; F, Fixation index.

Table 2. List of private alleles found in Ghoongroo pigs

Locus	Specific alleles observed
S0155	C(148), E (154)
S0005	J (245),K (247), M (251),N (253), O(255), U (267)
Sw2410	A (92), B (102), E (108), F (112), L (130)
S0355	G (264)
Sw24	A (93), D (99), G (109), J (117), L (124)
Sw632	B (152), D (156), J (172)
Swr1941	C (206), H (220)
S0218	H (174), J(178)
S0097	D (220), G (240)
IGF1	A (193)
Sw2406	E (230), J (246)
Sw72	F (109)
S0226	A (180), C (186),J (204), L (210)
S0068	A (211), C (215), E (219), F (221), G(223), N (238), P(242), Q (246)
S0178	A (101)
Total	45

determined using suitable decision support system (may be with molecular genetic information) to reduce the pressure on taxpayers' pockets. In this context, 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 and Ghoongroo was one among them which makes this study more important.

Genetic variability of microsatellite loci: A total of 180 alleles were observed in the 22 microsatellites; polymorphisms at all loci were observed in the examined population. 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 1. The allele size range varied from 89–103 bp at locus S0026 to 180–210 at locus S0226. The total number of alleles ranged between 3 (Sw2008) and 15 (S0068). It showed the genetic diversity within the population as assessed by effective number of alleles and heterozygosity. The effective number of alleles ranged from 2.2 (S0026) to 6.75 (S0068) with a mean of 4.39±0.026. The mean observed heterozygosities are lower than the expected values based on these 22 studied loci. The observed and expected heterozygosities ranged from 0.4 to 0.97 and 0.55 to 0.85 in Ghoongroo breed of pig, respectively. 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, high genetic diversity was reported earlier in *desi* and Gahuri pigs (Behl *et al.* 2002) and also in Chinese and Mexican pig populations (Lemus Flores *et al.* 2001, Fang *et al.* 2005). A total of 45 private alleles were described (Table 2) which was exclusive to a particular breed which can be used for breed paneling by molecular methods.

Polymorphic information content (PIC): PIC, a parameter indicative of the informative degree of a marker, for all the 22 markers is shown in Table 1. The PIC value can range from 0 to 1, however, range of PIC in this study was from 0.55 (S0026) to 0.85 (S0068) with the average value 0.75±0.02. All 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 6 highly polymorphic SSR loci, viz. *S0005, Sw 24, Sw 72, S0226, S0068* and *S0143* based on the parameters like PIC value, gene diversity, and polymorphic alleles. These 6 polymorphic primers can effectively be used in further 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.

Festimates: Inbreeding coefficients for all markers are given in Table 1. Average F value for markers ranged from

0.002 (Sw2410) to 0.43 (S0101). Among the negative values it ranged from –0.35 (S0026) to –0.02 (Sw830). The mean F value was 0.07, which indicated the nominal amount of inbreeding in the population. 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.

Genetic bottleneck analysis: Populations that have experienced a recent reduction of their effective population size exhibit a correlative reduction of the allele numbers and gene diversity at polymorphic loci. But the allele number is reduced faster than the gene diversity. Thus, in a recently bottlenecked population, the observed gene diversity is higher than the expected equilibrium gene diversity which is computed from the Na, 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 tests were used and 3 statistical tests are performed for each mutation model and the allele frequency distribution was established 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 Ghoongroo pig using 3 tests (IAM, TPM and SMM) are presented in Table 3. In Ghoongroo pigs, under Sign test, the expected numbers of loci with heterozygosity excess were 13.1 (IAM), 13.1 (TPM) and 13.0 (SMM). The 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) 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 0.000, 0.16 and 0.99 for IAM, TPM and SMM; these were significant (P<0.05) in case of IAM only.

Table 3. Population bottle neck analysis in native Indian pigs

Models	I.A.M.	T.P.M.	S.M.M.	
Sign test (No. of loci with heterozygosity excess)	Exp	13.1	13.1	13.0
	Obs	22.0	13.0	5.0
	P- value	0.1×10^{-4}	0.5	0.5×10^{-3}
Standardized differences test	T2 values	3.4	0.8	–3.9
	P- value	0.4×10^{-3}	0.2	0.5×10^{-4}
Wilcoxon test (one tail for H excess)	P- value	0.00	0.16	0.99

IAM, Infinite allele model; TPM, Two-phased model of mutation; SMM, stepwise mutation model.

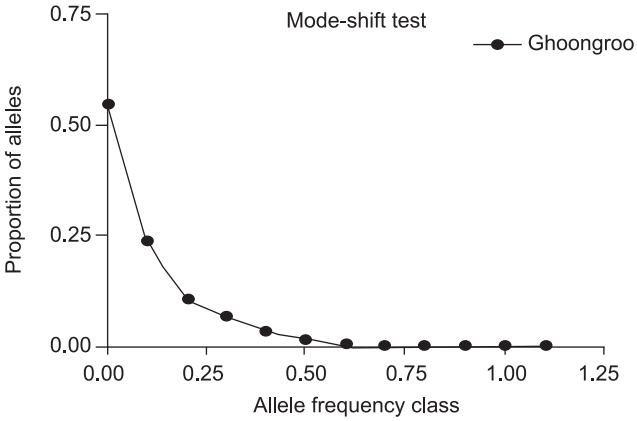

Fig. 2. Mode-shift test for bottleneck in Ghoongroo pigs.

In conclusion, the panel of microsatellites evaluated in Ghoongroo pig of India in the present study and showed high heterozygosity and polymorphism. This study showed that there is an abundance of genetic variation stored in this pig breed. The present study clearly verified that using these panel of microsatellites markers, different breeds or populations of native Indian pigs can be suitably investigated for the relationships and genetic diversity.

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