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Genetic diversity analysis of Ghurrah pig based on microsatellite markers

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Piggery sector acts as the backbone of tribal and rural economy. Majority of Indian pig population is non-descriptive (76% as per 2012 Census), locally adapted and reared by marginal and small farmers. They show diversified morphology and relatively lower productive and reproductive performance (Sahoo *et al.* 2012, Boro *et al.* 2016). Despite, having poor growth rate and lower feed conversion efficiency, these desi pigs have unique features such as better heat tolerance, meat quality, early sexual maturity (Kumaresan *et al.* 2008, Karunakaran *et al.* 2009) and quality bristles (Mohana *et al.* 2014) compared to exotic/crossbreds which indicates their potential for conservation and improvement. The primary objective of a conservation programme is to preserve as much genetic diversity as possible which requires precise evaluation and breed characterization (Boettcher *et al.* 2010). Ghurrah breed of indigenous pig has been registered as 8th pig breed of the country which makes this study more important.

The study included 40 Ghurrah pigs, which were selected from the breeding tract. The native pigs, which looked alike and lacked the history of crossbreeding, were selected from their breeding tracts as per the phenotypic breed characteristics mentioned in breed descriptor of NBAGR. To ensure un-relatedness, only 2 pigs from each village were sampled for the study. Sixteen pairs of primers (S0026, S0005, Sw2410, Sw830, Sw632, Swr1941, Sw122, IGF1, Sw2406, Sw72, S0226, Sw2008, S0101, S0143, S0068, S0178) gave good amplification. All animals were genotyped for those 16 fluorescence-labeled microsatellite markers amplified in four multiplex PCRs.

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. The multiplex-PCRs were performed in 25 µL volume containing 50 to 100 ng of porcine genomic DNA as template, 1× PCR buffer, 0.1 to 1.0 mM of forward (fluorescence labeled with FAM, VIC, NED and HEX) and reverse primers, 200 mM of each dNTP, 1× Green Tag buffer and 1 U of Taq polymerase. The reactions were performed on the thermal cycler under the thermal cycle profile: denaturation at 95°C for 10 min in the first cycle, 35 cycles of 95°C for 45 sec, 56–63°C for 45 sec, 72°C for 45 sec, and extension at 72°C 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 (ABI 3730 DNA Analyzer, Applied Biosystems, USA). The fragment size was calibrated with Peak Scanner Software version 1.0 (ABI PRISM, Applied Biosystems, USA).

Various measurements of within breed genetic variations, viz. observed number of alleles (Na), effective number of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He) for each locus 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 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, three tests, namely 'sign test', 'standardized differences test' and 'Wilcoxon sign-rank test' were employed.

Alleles (136) were observed in the 16 microsatellites; polymorphisms at all loci were observed in the examined population.

The allele size varied from 88 to 96 bp at locus S0026 to 203–243 at locus S0005. The total number of alleles ranged from 4 (Swr 1941 and SO143) and 21 (SO005). The genetic diversity within the population is explained by effective number of alleles and heterozygosities at different loci. The effective number of alleles ranged from 1.34 (Swr1941) to 11.51 (SO005) with a mean of 5.01±0.57. The mean observed heterozygosities are lower than the expected

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Table 1. Population genetic variability in Ghurrah pig

ISAG Locus	Na	Ne	Ho	He	Allelic range	PIC	F
S0026	5	4.42	0.61	0.773	88–96	0.75	0.21
S0005	21	11.51	0.73	0.91	203–243	0.91	0.21
Sw2410	8	4.64	0.60	0.78	104–122	0.78	0.24
Sw830	10	4.33	0.31	0.77	178–202	0.76	0.60
Sw632	10	3.73	0.55	0.73	148–168	0.73	0.25
Swr1941	5	1.34	0.15	0.26	202–216	0.25	0.40
Sw122	8	5.26	0.48	0.81	107–121	0.81	0.41
IGF1	7	5.30	0.43	0.81	195–207	0.81	0.47
Sw2406	13	6.02	0.62	0.83	223–235	0.83	0.26
Sw72	6	4.03	0.41	0.75	100–112	0.74	0.45
S0226	4	3.07	0.27	0.67	192–198	0.66	0.60
Sw2008	5	2.38	0.33	0.58	95–103	0.56	0.42
S0101	8	5.18	0.45	0.81	200–216	0.80	0.45
S0143	9	4.09	0.56	0.76	151–167	0.75	0.26
S0068	9	4.95	0.32	0.80	219–239	0.80	0.60
S0178	8	6.29	0.62	0.84	108–124	0.84	0.27
Mean±SE	8.50±0.99	4.78±0.57	0.47±0.04	0.74±0.05	–	0.74±0.04	0.38±0.12

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

values based on these 16 studied loci. The observed and expected heterozygosities ranged from 0.15 to 0.73 (0.47±0.04) and 0.17 to 0.91 (0.71±0.05) in Ghurrah breed of pig, respectively. The variations in genetic diversity of 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 indigenous pigs (Behl *et al.* 2002, Sahoo *et al.* 2015, Sahoo *et al.* 2016a,b) of India and also in Chinese and Mexican pig populations (Lemus Flores *et al.* 2001, Fang *et al.* 2005).

PIC value in this study varied from 0.25 (SwR1941) to 0.91 (SO005) with the average value 0.74±0.04. All the markers had PIC values higher than 0.5 (except SwR1941), 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 7 highly polymorphic SSR loci, viz. S0005, SO026, SO178, Sw2406, SO097, SO226 and SO068 based on the parameters like PIC value, gene diversity, and polymorphic alleles. These 7 polymorphic primers can be effectively used in further molecular breeding programs since they exhibited very high polymorphism over other loci.

F value for markers ranged from 0.21 (SO005) to 0.60 (Sw830, SO143 and SO068). The mean F value was 0.38±0.12 which indicated the amount of inbreeding in the population. The higher F indicated presence of low heterozygosity suggesting that these populations might have been managed under uncontrolled mating system leading to mating between the close relatives and increase in inbreeding co-efficient.

Under Sign test, the expected numbers of loci with heterozygosity excess were 9.54 IAM (Infinite Allele Model), 9.52 TPM (Two-phased model) and 9.41 SMM (Stepwise Mutation Model). Heterozygosis excess under TPM and SMM were 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 16 microsatellite loci as per earlier recommendations of 8–10 loci (Spencer *et al.* 2000) showed the normal L-shaped curve (Fig. 1). 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 0.001, 0.09 and 0.92 for IAM, TPM and SMM, respectively, which indicates the significant (P<0.05) value in case of IAM only.

The panel of microsatellites evaluated in Ghurrah pig of India in the present study showed moderate heterozygosity and polymorphism. The present study clearly verified that

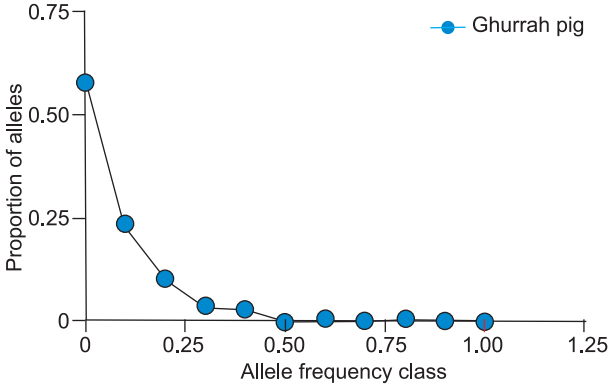

Fig. 1. L-shaped curve obtained for qualitative method of estimation of bottleneck.

using this panel of microsatellites markers, different breeds or populations of native Indian pigs can be suitably investigated for the relationships and genetic diversity.

SUMMARY

For analysis of genetic variation within Ghurrah, the local pigs of Rohilkhand region was explored using 16 FAO-ISAG microsatellite markers with 40 genetically unrelated pigs from the native breeding tract. Genomic DNA was isolated and amplified with microsatellite primers labeled with fluorescent dyes and genotyped using genetic analyser. The estimates of various genetic diversity parameters revealed mean number of observed alleles (Na), effective number of alleles (Ne), observed (Ho) and expected (He) heterozygosity values, polymorphic information content (PIC) and F-values to be 8.5±0.99, 4.78±0.57, 0.47±0.04 and 0.74±0.05, 0.74±0.04 and 0.38±0.03, respectively. The high PIC value suggested that all the microsatellite markers were highly polymorphic and suitable for molecular characterization of this pig. There was substantial genetic variation and polymorphism across the studied loci. There was absence of genetic bottleneck in the studied population. The medium inbreeding coefficient indicates a need to formulate the appropriate breeding strategies to enhance heterozygosity in the population.

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