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Original Research Article

Molecular characterization of Ghungroo pig

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Abstract

Ghungroo pig is indigenous to West Bengal and has already made in road to NE India for its high productivity. A total of 21 microsatellite markers were used to study genetic diversity and population structure of Ghungroo pig. All the considered loci were highly polymorphic and a total of 103 alleles were observed across the studied loci. The range of alleles was found to be 2 to 9 with a mean of 4.9±2.567. The frequency distribution of microsatellite alleles in the population was from 0.0179 to 0.9615. The calculated observed and expected heterozygosity values were 0.55±0.356 and 0.58±0.216 respectively. The PIC was 0.54±0.22 and Shannon's information index was sufficiently high with a mean of 1.16. The overall mean of within-population inbreeding estimate (F_{IS}) was 0.0919. The bottleneck analysis revealed that population has not undergone any recent reduction.

Keywords: *Ghungroo pig, Heterozygosity, Microsatellites, PIC*

1. Introduction

Ghungroo pig (GP) an indigenous and potential strain first reported from North Bengal. The Ghungroo are mostly black in color with typical bull dog face appearance however, docile in nature (Fig. 1). The average litter size of GP is 9-12, individually weigh about 6-8 kg at weaning and it is mainly reared by the tribes of Duars Valley and associated zone in the West Bengal state of India. This pig is popular because of high prolificacy and ability to sustain in low input system. In the breeding tract they are maintained under scavenging system and mainly act as insurance to the rain-fed agriculture.

To date no genetic diversity studies are documented on GP population using microsatellites. Considering the importance and utility the present study has been planned to investigate genetic variation and population structure within GP population using 21 polymorphic microsatellite markers.

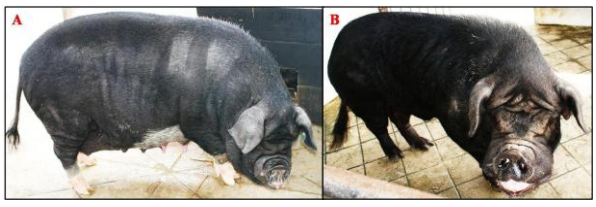

Fig. 1 Typical Ghungroo pigs in organized farm (A) Sow and (B) Boar

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2. Materials and methods

A total of 40 blood samples of GP were collected randomly from genetically unrelated individuals from their native breeding tract (Fig 2). Blood was collected aseptically into BD vacutainers (6 ml) containing K2 EDTA (10.8 mg) and samples were transported to the laboratory on ice and were stored at 4°C until use. Genomic DNA was extracted from the blood samples using standard phenol-chloroform method (Sambrook *et al*, 1989) with few modifications. The quantity and quality of isolated DNA were confirmed. The concentrated samples were diluted to reach appropriate concentrations (20-50 ng/µl) for the purpose of PCR amplification.

Fig. 2 Figure showing the breeding tract of GP (Kindness to Google earth map)

Table 1 Microsatellite analysis in Ghungroo pig (GP)

Panel	Locus	Size range(bp)	Parameters N_a	N_e	PIC	H_o	H_e	I	F_{IS}	HWE
Panel 1	SW 936	91-111	9	5.1579	0.7835	0.9643	0.8061	1.8708	-0.1962	136.22**
	SW353	141-165	3	1.2897	0.2096	0.0625	0.2246	0.4471	0.7217	84.93**
Panel 2	TNFB	155-173	3	1.5556	0.3254	0.2857	0.3571	0.6560	0.2000	20.74**
	SW24	102-104	2	1.6575	0.3180	0.3636	0.3967	0.5860	0.0833	0.2^{NS}
	S0355	243-255	6	3.9765	0.7060	0.6154	0.7485	1.5116	0.1779	45.92**
	S0107	187-201	9	6.4433	0.8273	0.9600	0.8448	2.0154	-0.1364	14^{NS}
Panel 3	SW72	106-114	5	2.8481	0.5939	1.0000	0.6489	1.2244	-0.5411	8.4*
Panel 4	S0228	223-245	9	5.2093	0.7833	0.9286	0.8080	1.8614	-0.1492	59.25^{NS}
	SW122	100-136	3	1.8113	0.3967	0.2500	0.4479	0.7781	0.4419	10.12^{NS}
Panel 5	S0008	220-236	5	3.1613	0.6350	0.5000	0.6837	1.3179	0.2687	14.66**
	SW957	118-126	3	2.6667	0.5546	0	0.6250	1.0397	1.0000	3^{NS}
	S0225	183-197	3	1.5728	0.3266	0.1111	0.3642	0.6547	0.6949	2^{NS}
	S0010	100-124	2	2.0000	0.3750	1.0000	0.5000	0.6931	-1.0000	31.33^{NS}
Panel 6	S0070	273-283	2	2.0000	0.3750	1.0000	0.5000	0.6931	-1.0000	11.48**
	SW911	159-171	6	3.7029	0.6903	0.6111	0.7299	1.4749	0.1628	31.89^{NS}
	S0086	162-178	4	1.9048	0.4400	0.1000	0.4750	0.9143	0.7895	26.65^{NS}
Panel 7	S090	241-251	5	3.4595	0.6677	0.6250	0.7109	1.3903	0.1209	36.33**
	IGFI	216-236	5	4.8358	0.7600	0.7778	0.7932	1.5918	0.0195	64.36**
Panel 8	S0386	167-185	9	4.7108	0.7653	0.6923	0.7877	1.8141	0.1211	6^{NS}
Panel 9	CGA	250-308	2	1.0799	0.0712	0.0769	0.0740	0.1630	-0.0400	130.11**
Panel 10	S0226	193-197	8	4.1858	0.7324	0.6154	0.7611	1.6845	0.1914	0.02^{NS}
Mean overall loci			4.90 ± 2.567	3.15 ± 1.546	0.54 ± 0.220	0.55 ± 0.356	0.58 ± 0.216	1.16 ± 0.544	0.0919	

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 excess of heterozygotes, HWE, Hardy-Weinberg equilibrium; I, Shannon's Information Index .
* Significant ($P \leq 0.05$); **Highly significant ($P \leq 0.01$); ^{NS} Not significant ($P \geq 0.05$).

Table 2 Bottleneck analysis in Ghungroo pig (GP)

Model	Sign rank test - Number of loci with heterozygosity excess			Standardized differences test - T2 values (probability)	Wilcoxon test - Probability of heterozygosity excess
	Expected	Observed	Probability		
IAM	11.61	16	0.04769	2.350 (0.00940)	0.01079
TPM	12.11	12	0.56384	0.847 (0.19855)	0.18686
SMM	12.24	7	0.01839	-1.198 (0.11543)	0.86389

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

A total of 21 microsatellite markers were selected for present study and the forward primer of each marker was fluorescently labeled with either FAM, NED, PET or VIC dye. All microsatellite markers were first checked under single locus amplification conditions to evaluate their performance in the multiplex.

Multiplex PCR has been used for multicolor fluorescence genotyping. Based on the guidelines of Henegariu *et al,* (1997) and Loffert *et al,* (1999) the initial parameters of multiplex PCR were set up. The basic PCR reaction mixture (15 µl) containing 20-50 ng of template DNA; 1.5 mM $MgCl_2$; 5 picomoles 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 (54°C to 60°C for 30 sec) and extension (72°C for 45 sec). PCR conducted on an Applied Biosystems (Model #: 9902) Veriti^{TM} 96- well thermal cycler. Genotyping was carried out on an automated DNA Sequencer (ABI PRISM 3130XL). The resulting data were analyzed using standard 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.

POPGENE (version 1.31) (Yeh *et al,* 1999) was used to calculate the allele frequencies, effective number of alleles (N_e), observed (H_o) and expected (H_e) heterozygosity, F-statistics, Shanon's information index (I) and to test of Hardy-Weingberg equilibrium (HWE). Nei's formula (Nei, 1978) was used to calculate polymorphic information content (PIC). The BOTTLENECK (version 1.2.03) (Cornuet and Luikart, 1996) analysis was performed to know whether this pig population exhibits a significant number of loci with excess of heterozygosity.

3. Results

The various parameters of genetic differentiation in GP, 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.

The number of observed alleles (N_a) detected ranged from 2 (SW24, S0010, S0070 and CGA) to 9 (SW936, S0107, S0228 and S0386), with an overall mean of 4.90±2.567 and a total of 103 alleles were observed at these loci in the population. However, the effective number of alleles (N_e) ranged from 1.0799 (CGA) to

6.4433 (S0107) with a mean of 3.15±1.546. Overall allele frequency ranged from 0.0179 (at loci SW936 and S0228) to 0.9615 (at locus S0226). The PIC value ranged from 0.0712 (CGA) to 0.8273 (S0107) with a mean of 0.54±0.220. The overall means for observed (H_O) and expected (H_e) heterozygosities were 0.55±0.356 and 0.58±0.216 respectively which ranged from 0 (SW957) to 1 (SW72, S0010 and S0070) and 0.074 (CGA) to 0.844 (S0107) respectively.

The chi-square (χ^2) test for HWE revealed that 10 out of 21 loci deviated from equilibrium. Shannon's information index (I) which measures the level of diversity, was sufficiently high with a mean of 1.16±0.544. The within population inbreeding estimates (F_{IS}) observed at 14 loci were positive and 7 loci revealed negative with a overall mean of 0.091 indicating that only 9.1 per cent of inbreeding was recorded in GP population. The three mutation models namely, infinite allele model (IAM), two phase model (TPM), stepwise mutation model (SMM) were calculated using the programme Bottleneck and results are presented in Table 2. The mode shift analysis i.e. qualitative method of estimation of bottleneck showed the normal L-shaped curve (Luikart *et al,* 1998) in the present study, no mode-shift was detected in the frequency distribution of alleles. The graphical representation of mode-shift was shown in Fig. 3.

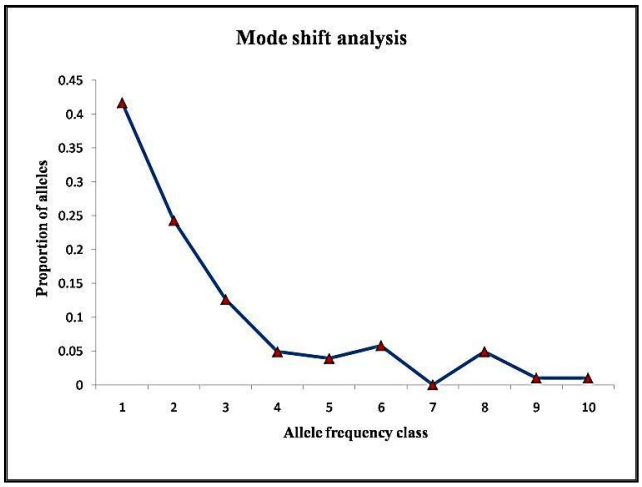

Fig. 3 Figure showing the graphical representation of allele proportions and their contribution in GP

4. Discussion

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 (FAO 1998). The mean number of alleles observed (4.9) in the study is less than the mean number reported for North Indian (7.92), Northeast Indian pig (7.84) types (Rajeev Kaul *et al,* 2001) and Brazilian (8.96) pig breeds (Sollero *et al,* 2010). However, the mean number of effective alleles (3.15) is higher than the mean number reported Brazilian pig (Sollero *et al,* 2010) breeds Landrace (2.70), Monterio (2.34), Moura (2.32), MS60 (2.56) and Piau (2.94). This pig population showed that low effective number of alleles than the observed number of alleles 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.

In Brazilian pig breeds (Sollero *et al,* 2010) using 28 different microsatellite markers reported the range of PIC between 0.137 and 0.874 with the mean of 0.655 which is in close agreement with the present findings which ranged from 0.0712 to 0.8273 with a mean of 0.54. Most of the loci possessed high PIC values signifying that these markers are highly informative for characterization of GP. Thus these markers strongly signified genetic diversity investigations of GP.

The mean of observed and expected heterozygosity (0.55 and 0.58) in the study corroborates with the mean number of observed (0.58) and expected (0.68) heterozygosity in Brazilian pig breeds (Sollero *et al,* 2010) and also observed heterozygosity found in present study is comparable to the Southern African domestic (Swart *et al,* 2010) pigs Landrace (0.522); Large White (0.584); Duroc (0.504); Namibia (0.518); Mozambique (0.609); Kolbroek (0.537) and Kune-Kune (0.508).

The mean within population inbreeding estimate (F_{IS}) was 0.091. The deficiency of heterozygotes (9.1 per cent) in GP population in accordance to heterozygote shortfall observed in Duroc pig (5.1 per cent); Landrace pig (3.8 per cent); Large White pig (6.5 per cent); Pietrain pig (6.1 per cent) (Vicente *et al,* 2008) and not significant as compared to heterozygote shortfall reported in Bae pig (22.6 per cent); Canastra pig (23 per cent); UDB pig (22.8 per cent); Duroc pig (25 per cent) (Silva *et al,* 2011). The present findings of F_{IS} value supports random mating in the studied population. The deviation of 10 out of 21 loci from equilibrium may be due to consequences of small population size.

Bottleneck programme was used to evaluate three mutation models namely, infinite allele model (IAM), two phase model (TPM), stepwise mutation model (SMM) in GP population. The population has not undergone any recent and/or sudden reduction in the effective population size and remained at mutation-drift equilibrium. In the present study, no mode-shift was detected in the frequency distribution of alleles and a norm al L-shaped curve was observed.

5. Conclusion

The study stands first in genetic characterization of GP through microsatellite markers. The results revealed that the polymorphic nature of microsatellite loci screened in GP population. The significant level of variability in this population is indicative of valuable genetic diversity. The population has not undergone any reduction at least in the recent past revealed that scope for further development. The needful strategy steps to be taken to maintain the existing genetic variation and its sustainable utilization.

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