GENETIC DIVERSITY OFWILD BOAR AND VILLAGE PIGS IN SRI LANKA

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ABSTRACT

A study was conducted to understand molecular genetic characteristics of village pigs and wild boar populations using microsatellite markers. A total of 15 microsatellite markers recommended by FAO/ISAG (Food and Agriculture organization/International Society for Animal Genetics) were employed in this molecular investigation. Among 15 microsatellite loci 11 were polymorphic and rests were monomorphic. The total observed number of alleles per locus varied from 2 to 4 in all the populations. The mean effective number of alleles for 15 loci in wild boar, village pigs and exotic types were 2±0.38, 2±0.53 and 1.73±0.59 respectively. The observed heterozygosity value was higher in village pigs (0.72±0.02) than that in other pig populations used in this study indicated that the village pig population of Sri Lanka showed a high genetic diversity compared to the other pig populations including exotic pigs and wild boar in Sri Lanka.

The phylogenetic tree was constructed using 1000 bootstrap values for the individuals in populations of village pig and wild boar indicated that there was a high genetic variation among individuals in both village pig and wild boar populations. The clustering pattern of phylogenetic consensus tree further revealed that there were unique group of wild boar and village pigs. However, the result of this study showed that the village pigs in certain geographic areas of the country has closer genetic relationship with wild boars. This observation could be very well confirmed by the breeding practice of village pigs in those areas of the country. And also on the basis of these results, it is evidenced that both village pigs and wild boar need equal attention in conservation attempts. However, it is advisable to expand the comparison with other native pig types of the region before coming to conclusion on conservation program.

Key words : Village pigs, Wild boar, Microsatellite markers, Heterozygosity, Genetic diversity, Phylogenetic tree

INTRODUCTION

The village pigs have long been reared as backyard scavengers in the Western Coastal belt of Sri Lanka. In Sri Lanka, the village pigs are popular for their quality and tasty meat. There is no planned breeding program for the improvement of village pigs in Sri Lanka and as a result the population is decreasing gradually. Despite decreasing trends in populations the native breed still comprises a valuable component of local genetic resources. The village pig closely resembles the Sri Lankan wild pig and must have evolved as a result of gradual domestication of wild pigs, although studies on its phylogeny have yet to be undertaken (Rajamahendran *et al*., 1978). Their genetic diversity has provided the material for the very successful pig breeding and improvement programs of the developed world in the 19th and 20th century.

There are several attempts made in the recent past to evaluate growth and reproduction parameters of village and wild type pigs in Sri Lanka. However, the existing genetic diversity in wild boars and village pigs has not been subjected to comprehensive scientific evaluation. In this context, a study was formulated to identify the genetic diversity of wild and village pigs in Sri Lanka with the aim of generating information to establish genetic improvement programfor native pigs to facilitate their contribution in rural economy and nutrition.

MATERIALS AND METHODS

Bloodsamplecollection and isolationof genomic DNA

Bloodsamples were collected fromKalutara, Kurunagala, Puttalam and Chilaw for village pigs and Batticaloa, Polonnaruwa, Trincomalee, Anuradhadapura, Kandy and Kurunagala for wild boar. Twenty unrelated village pigs were sampled from several farm holdings from selected areas and similar numbers of wild boars were sampled from different slaughtering places from selected areas. Five milliliter of blood from ear vein was collected from village pigs and exotic pigs.

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The genomic DNA was extracted from the collected blood samples using salting out protocol. Quantification of DNA was done using a spectrophotometer (UV visible spectrometer, model-cintra 10e). Genomic DNA was amplified by polymerase chain reaction using the ISAG/FAO (International Society for Animal Genetics/ Food and Agriculture Organization) recommended microsatellite primers (S0355, S0218, S0228, S0226, S0090, SW2008, SW1067, SW911, SW256, SW 1089, SW 310, SW 252, SWR198, SW335 and SW122). Primers were synthesized by TIBMOLBIOL, Germany. Each 30 µl reaction consisted of DNA (50 ng/µl), primers (20µM each), dNTPs (ABgene, UK 2.5mM each), 2.5 ml of 10 × buffer (10 mM Tris, 50 mM KCl, 0.1% gelatin; pH 8.4), $MgCl_2$ (1.5 mM) and *Taq* DNA polymerase (ABgene, UK 5 unit/µl)). The thermocycle conditions were: 5 minutes at 94°C followed by 30 cycles of 45 seconds at 94°C, 45 seconds at annealing temperature and 1 minute at 72°C. The last elongation step was prolonged to 10 minutes. The reaction was stopped at 4°C. Amplified DNA fragments were analysed on 6% denaturing polyacrylamide gel and detected by silver staining. The heterozygosity or genetic diversity was calculated according to Nei (1978), the observed and expected heterozygosity and effective numbers of alleles were calculated using the MICROSTAT computer package (version 1.5 d).

RESULTS AND DISCUSSION

Allele frequencies for the 15 microsatellites used in five populations consist of wild boar population, village pig population, and 3 exotic pig breeds (Landrace, Large White and Duroc) are presented in Table 1. In total, 138 alleles were found at the 15 loci screened across five populations in this study. Among 15 microsatellite loci 11 were polymorphic (Example, SW1067 and SW355) and rests were monomorphic (Example, SW911 and SW335).

The polymorphic alleles were ranged between 2 alleles per locus to 4 alleles per locus. Loci S0090, S0228, SW218 and SW 226 carried unique alleles (at 242, 220, 218 and 202 bp, respectively) for the three exotic breeds tested. In the case of village pigs loci S0090 (allele 144 and 262), SW218 (allele 206 and 212) and SW 226 (alleles 198 and 218) showed uniqueness. Wild boars carried two rear alleles (found in only few wild boars) among the tested loci; locus SWR198 (allele 122) and locus SW 335 (allele 98). Village pigs carried only one rear allele, which was at S0228 locus (allele 166). However, no rear alleles were found among the three exotic breeds studied (Table 1). The mean effective number of alleles for 15 loci in wild boar, village pigs and exotic types were 2±0.38, 2±0.53 and 1.73±0.59 respectively (Table 2). The observed number of alleles and their frequencies indicate that wild boar and village pigs are having high allelic richness compared to exotic pig types.

The average observed heterozygosities are higher than the expected values of the 15 loci in all the populations (Table 2). The observed heterozygosity was comparatively high in the native pig populations. This may be due to low selection pressure in the village pig and wild boar populations. The genetic diversity within the population as assessed by effective number of alleles and heterozygosity was lower in village pigs and wild boar when compared with various other studies on European native pig breeds. (Fredholm *et al.,* 1993; Laval *et al.*, 2000; Martinez *et al.*, 2000, Van Zeveran *et al.,* 1995). The higher genetic diversity levels present in European native pig breeds may be the result of large effective populations when compared with European pig breeds, which are well-defined pure-bred stock represented by smaller populations. However, in Sri Lanka the size of village pigs as well as wild boar populations are very low compared to those in above mentioned countries. This may be the reason for the low genetic diversity, observed in village pig and wild boar populations in the present study.

The observed heterozygosity value was higher in village pigs (0.72±0.02) than that in other pig populations used in this study (Table 2). This result indicates that the genetic diversity is comparatively higher in village pig population than other pig populations. In addition, the higher heterozygosity may be due to the free range mating system and lack of directional selection. Wild boar population showed the genetic diversity of 0.64±0.02. The heterozygosities observed in the Sri Lankan wild boar and village pig populations are similar to those observed in native Indian pigs (0.56±0.07 to 0.74±0.09) (Rajeev *et al*, 2001). The lower genetic diversity of exotic pigs is expected given the purebred condition generally adopted in the process of developing commercial breeds.

The phylogenetic consensus tree constructed using the neighbor joining method groups the individuals

Table 1 : The allele frequencies at 15 microsatellite loci in wild boar (WB), village pigs (VP), Landrace (LR), Large White (LW) and Duroc (D) pigs

Locus	Allele size	Allele frequency				
		WB	VP	LW	LR	D
S0090	144	0.92	0.75	0	0	0
	242	0	0	1	1	1
	262	0.08	0.25	0	0	0
S0228	166	0	0.05	0	0	0
	220	0	0	0.6	0.2	0.6
	222	0.5	0.48	0.2	0.4	0.2
	226	0.5	0.48	0.2	0.4	0.2
SW122	97	0.5	0.55	0.6	0.5	0.5
	131	0.5	0.45	0.4	0.5	0.5
SWR198	96	0.92	1	1	1	1
	122	0.08	0	0	0	0
SW218	206	0.23	0.13	0	0	0
	212	0.77	0.87	0	0	0
	218	0	0	1	1	1
SW226	178	0.5	0.5	0.5	0.5	0.5
	198	0.25	0.13	0	0	0
	202	0	0	0.5	0.5	0.5
	218	0.25	0.37	0	0	0
SW252	94	0.85	0.65	0.9	1	1
	161	0.15	0.35	0.1	0	0
SW256	97	0.6	0.5	0.5	0.5	0.5
	130	0.4	0.5	0.5	0.5	0.5
SW310	97	0.5	0.53	0.7	0.8	0.9
	149	0.5	0.47	0.3	0.2	0.1
SW335	104	0.5	0.5	0.5	0.5	0.5
	114	0.5	0.5	0.5	0.5	0.5
SW355	98	0.07	0	0	0	0
	102	0	0.28	0	0.1	0.2
	242	0.93	0.72	1	0.9	0.8
SW911	151	0.5	0.5	0.5	0.5	0.5
	173	0.5	0.5	0.5	0.5	0.5
SW1067	98	0.68	0.5	0.5	0.5	0.5
	130	0.32	0.5	0.5	0.5	0.5
S02008	94	1	1	1	1	1
S01089	156	0.5	0.5	0.5	0.5	0.5
	78	0.5	0.5	0.5	0.5	0.5

Table 2 : Population parameters as determined by the distribution of 15 loci in five different populations

Population	Loci typed	Expected heterozygosity ±SD	Observed heterozygosity±SD	Mean number of alleles
Wild boar	15	0.38±0.04	0.64±0.02	2.00±0.38
Village pigs	15	0.41±0.04	0.72±0.02	2.00±0.53
Large White	15	0.34±0.06	0.53±0.05	1.73±0.59
Land Race	15	0.34±0.07	0.56±0.05	1.73±0.59
Duroc	15	0.33±0.06	0.53±0.05	1.73±0.59

SD - Standard Deviation

into four clusters (Figure 1). The first cluster had wild boar population while second cluster had wild boar and village pigs. All the exotic pigs found in fourth cluster. Most of the village pigs were found in third cluster. The clustering pattern revealed that some village pigs have similarities to wild boar (cluster 2). However, the result of this study showed that there were unique group of wild boar (cluster1) and village

pigs (cluster 3). The village pigs and wild boar included in this study clearly diverged from each other. It shows that the village pig and wild boar populations do not have a very close genetic relationship at present though might have originated from a common source.

The wild boar samples collected (WB6, WB9, WB12 and WB16) from Kurunagala area was found in cluster 2 which groups both village pigs and wild boars (Figure 1) The village pigs found in Kurunagala district (VP 20, VP22, VP 11, VP 14 and VP 15) had long straight face, upwardly erect ears, angular body shape and long hairs densely found along the spine. These traits are very similar to the wild boars in Sri Lanka. These morphological similarities suggest that the village pigs might have crossed with wild boar in this area as those village pigs were allowed to scavenge along neighboring forest sides where wild boars could be found.

This agrees with the report by Nozawa, 1980, who suggested that in rural areas where pigs are raised as free range, there is an opportunity for wild boars living in neighboring forest to mate with native pigs. The village pig samples collected from Kalutara (V17, V18, V19, V7, and V2) have also fallen in cluster 2. These village pigs also were with long straight face, upwardly erected ears and angular body. These morphological features are very similar to body characteristics of wild boar. In Kalutara district the village pigs are managed under extensive system, which allows free crossing with other animals including wild boars.

Most of the Village pig samples collected from Chilaw and Puttalam was grouped in cluster 3. In these areas pigs were kept under semi-intensive system of rearing (rearing in concrete pens and huts). This might limit mixing with other native pig populations. Exotic pig population grouped into completely different cluster (cluster 4) revealing the distinct genetic make up of those from the native pig types.

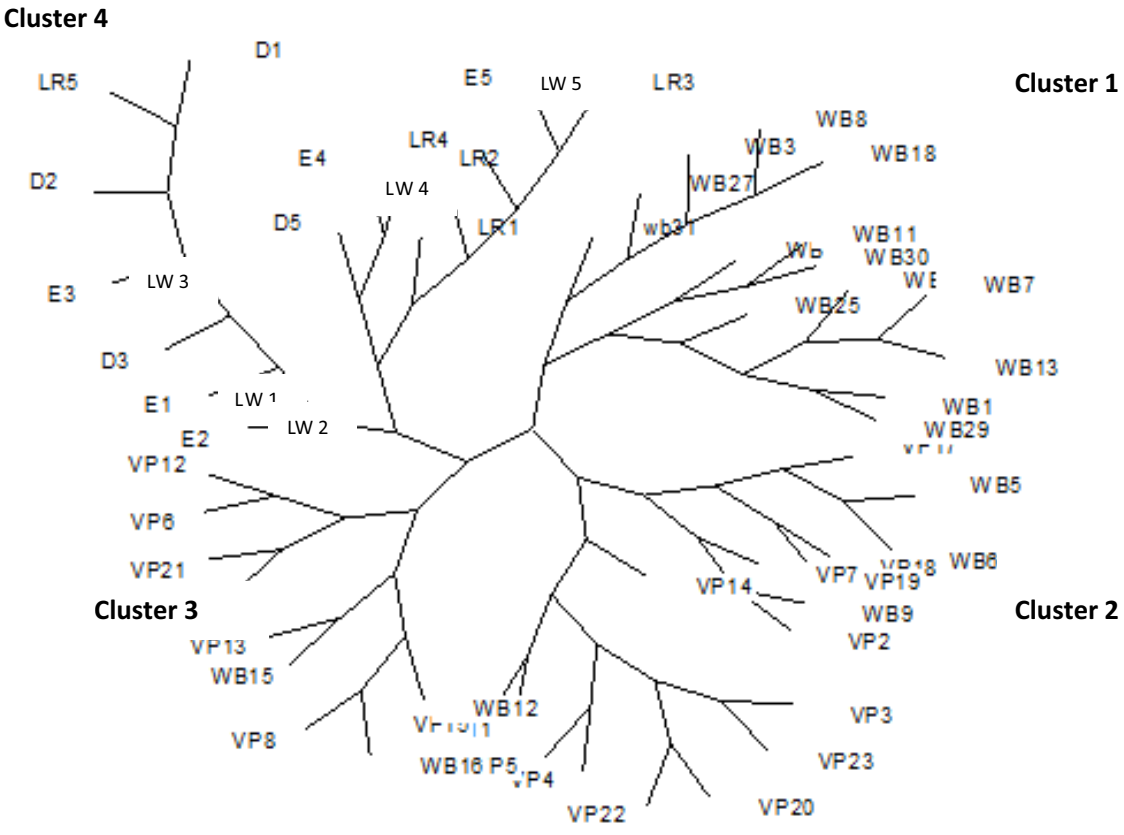

Figure 1: Dendrogram of genetic distances within village pigs and wild boar

CONCLUSION

The molecular findings revealed that village pigs and wild boars are two distinct populations however, the village pigs in certain geographic areas of the country has closer genetic relationship with wild boars. This observation could be very well confirmed by the breeding practice of village pigs in those areas of the country. The native pig population of Sri Lanka showed a high heterozogosity and a high genetic diversity compared to the exotic pig populations in Sri Lanka.

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