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Molecular Characterization of Native Swine Types in Sri Lanka

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ABSTRACT. *A study was conducted to understand the molecular genetic characteristics of native swine populations in Sri Lanka. A total of 15 microsatellite markers recommended by the Food and Agriculture Organization/International Society for Animal Genetics were employed in the molecular investigation. Among the 15 microsatellite loci, 11 were polymorphic and rest was monomorphic. The total observed number of alleles per locus varied from 2 to 4 in all swine 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 two pig populations. The phylogenetic tree constructed using 1000 bootstrap values for the individuals 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 groups of wild boar and village pigs. However, the result of this study also showed that the village pigs in certain geographic areas of the country have a closer genetic relationship with wild boars. This observation is very well justified by the breeding practice of village pigs in those areas of the country. The study concludes that the native swine populations in Sri Lanka are with unique genetic makeup and also with a high heterozogosity compared to the exotic pig populations in Sri Lanka.*

Keywords: *Native swine, microsatellite, heterozygosity*

INTRODUCTION

Of the total pig population in Sri Lanka more than 60 percent are low producing indigenous animals. Despite decreasing trends in populations the native pigs still harbor a considerable proportion of genetic resources. The village pigs are popular for their quality and tasty meat and closely resemble the Sri Lankan wild pig (*Sus scrofa)*. It is believed that the Sri Lankan village pigs have been evolved as a result of gradual domestication of wild pigs, although the proof from phylogeny is not available (Rajamahendran *et al*., 1978). The genetic diversity exist among wild populations have provided the material for the very successful swine breeding and improvement programs of the developed world in the 19th and 20th century.

In the absence of planned breeding program for the improvement of native swine breeds in Sri Lanka, these populations are decreasing gradually causing genetic erosion. The pig industry in Sri Lanka is mainly oriented towards the exotic germplasm replacing the most

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valuable genetic resource exists within the indigenous populations. Characterization of populations is a basic requirement for designing genetic improvement as well as conservation programs where animals need to be described morphologically (Subalini *et al*., 2010 and 2011) and genotypically (Hoffmann and Scherf, 2006) along with the systems of rearing or existence. The molecular characterization is considered as a more precise tool in describing populations with a good understanding in guiding the decision making for livestock development, breeding and conservation (Hanotte and Jianlin, 2005).

There have been several attempts made in the recent past to evaluate growth and reproduction parameters of native type of pigs in Sri Lanka including the phenotypic characterization (Goonewardena *et al.,* 1984*,* Sahaayaruban *et al*., 1984, Dematawewa *et al.,* 1999, Silva *et al.*, 1999; 2004). However, only a few reports are available on molecular level of characterization of wild and village type pigs in Sri Lanka. For example, protein polymorphism has been used to investigate the diversity among native pigs by Tanaka (1974). More recently a comparison of wild, domesticated and exotic types of pigs using protein polymorphism was reported by Keerthiratne (1998). However, these swine types have not been subjected to comprehensive scientific evaluation through molecular characterization.

In this context, the present study was done to investigate the genetic diversity exists among native pig populations and compare the relatedness among different pig populations. The study was carried out using microsatellite markers, which are the markers of choice for the detection of genetic diversity, especially in livestock due to their abundance, ubiquitous distribution, polymorphic nature, and suitability for amplification by polymerase chain reaction (PCR) (Bruford and Wayne, 1993). The study aims to generate data useful in planning genetic improvement program to facilitate their contribution in rural economy and nutrition, as well as designing the conservation program for their future utilization.

MATERIALS AND METHODS

A molecular investigation was done using microsatellite markers to characterize different swine populations in Sri Lanka including native and exotic swine types. The native pigs namely, village pigs and wild boars were sampled from various places in Sri Lanka. The exotic pigs were included in the study in order to complete the picture of the diversity exist in all populations of pigs in different origins. Hence, three exotic pig breeds commonly used in commercial pig production in Sri Lanka, namely Landrace, Large White and Duroc were evaluated. Laboratory investigations included mainly extraction of genomic DNA, quantification of DNA, Polymerase Chain Reaction and Polyacrylamide Gel Electrophoresis and silver staining.

Blood Sample Collection

Blood samples were collected from Kalutara, Kurunegala, Puttalam and Chilaw for village pigs and Batticaloa, Polonnaruwa, Trincomalee, Anuradhadapura, Kandy and Kurunegala for wild boar (Fig. 1). The twenty village pigs sampled were unrelated and from several farm holdings. A similar number of wild boars samples were collected from different slaughtering places. The blood samples of exotic pigs were collected from Swine Breeding Station at Welisara, Chutiduwa Swine farm at Puttlam and Livestock Experimental Station of Department of Animal Science, University of Peradeniya. Five milliliter of blood from ear vein was collected from village pigs and exotic pigs. Blood from wild boar was

collected during slaughtering and also from vein on hind limb after sedation of animal depending on the situation.

Blood sample was collected into vacutainer containing anticoagulant (200 µl of 0.129 mM Sodium citrate per 500 µl of blood) using fresh needle (18 gauge x 1.5 inches) for every animal under sterile conditions without cross contamination. The blood samples were transported on ice immediately to the laboratory and stored in 4°C until the analysis is carried out.

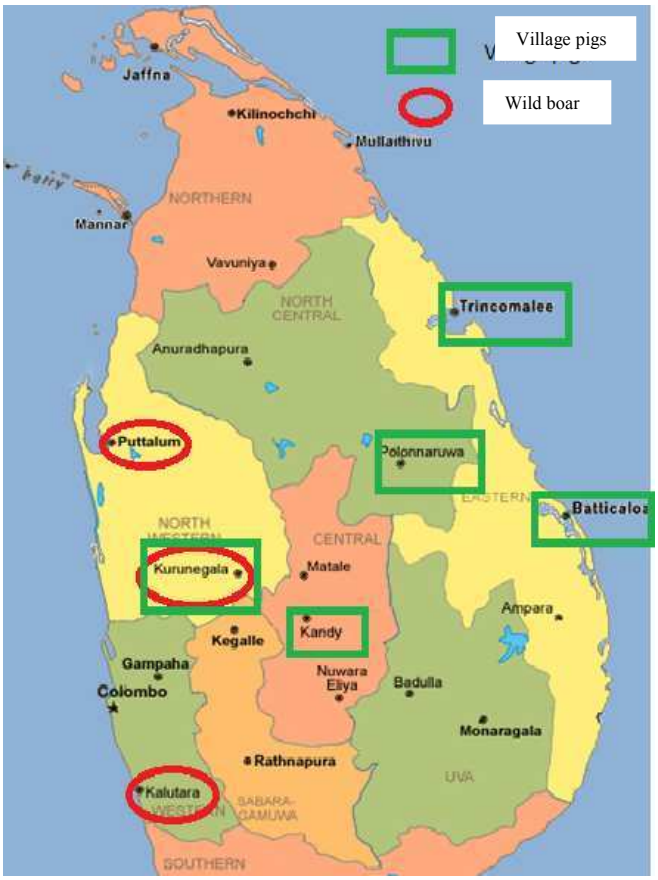

Fig. 1. Village pigs and wild boar sampling areas

DNA Extraction and Quantification

The genomic DNA was extracted from the collected blood samples using salting-out protocol (Miller *et al*., 1988). Quantification of DNA was done using a spectrophotometer (UV visible spectrometer, Model-cintra 10e). Agarose gel electrophoresis (0.8% agarose) was done to confirm the presence of DNA and samples were stored at -20°C.

DNA Amplification

Genomic DNA was amplified by polymerase chain reaction using microsatellite primers recommended by ISAG/FAO (International Society for Animal Genetics/ Food and Agriculture Organization) (Table 1). Primers were synthesized by TIBMOLBIOL, Germany. Each 30 µl reaction consisted of 4 µl template DNA (concentration : 50 ng/µl), 1.6 µl primers (20µM each), 2 µl dNTPs (ABgene, UK 2.5mM each), 3 µl 10 × buffer (10 mM Tris, 50 mM KCl, 0.1% gelatin; pH 8.4), 5 µl $MgCl_2$ (1.5 mM) and 0.3 µl *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 amplified products were stored at 4°C until visualization.

Table 1. Description of microsatellite markers used for analysis

Marker	Sequence (5'-3')	Annealing temperature	Allele range (bp)
S0355	F-TCTGGCTCCTACACTCCTTCTTGATG	50ºC	244-271
S0218	R- TTGGGTGGGTGCTGAAAAATAGGA	55 ºC	158-205
S0228	F- GTGTAGGCTGGCGGTTGT R- CCCTGAAACCTAAAGCAAAG	55 ºC	220-246
S0226	F-CAAAAAAGGCAAAAGATTGACA R-AGCCCACCTCATCTTATCTACACT	55 ºC	180-210
S0090	F-GCACTTTTAACTTTCATGATACTCC R-GGTTAAACTTTTNCCCCAATACA	55 ºC	227-249
SW2008	F-CCAAGACTGCCTTGTAGGTGAATA R-GCTATCAAGTATTGTACCATTAGG	55 ºC	95-108
SW 1067	F-CAGGCCAGAGTAGCGTGC R-CAGTCCTCCCAAAAATAACATG	55 ºC	136-176
SW911	F-TGCTGGCCAGTGACTCTG R-CCGGGGGATTAAACAAAAAG	60 ºC	149-173
SW256	F-CTCAGTTCTTTGGGACTGAACC R-CATCTGTGGAAAAAAAAAGCC	62 ºC	92-118
SW1089	F-ACAAAAGCTTTTGGAGAACTCG R-TAGCATAGGAACAGGTGCAGC	58 ºC	142-182
SW310	F-TTTTCCCCTTCACTCACCC R-GATCAAAGTCCCTTACTCCGG	62 ºC	109-135
SW252	F-CAGAAGGATGAATATGCAAAATG R-GTCTTTCAGGCTTGGAGGG	62 ºC	149-179
SWR198	F-CTCTGGGTCCATCCATTTTG R-TTATGATGCAAAACATGGAAGC	60 ºC	72-113
SW335	F-TTTCATCAGCAACTTCAGAAGG R-GGTGCGGCCCTAAAAAAAAG	55 ºC	100-112
SW122	F-GAGTATGGGGAAAAGCCACG R-CCATCAACAAACTGTATGCACC	55 ºC	106-128
	F- CAAAAAAGGCAAAAGATTGACA R- TTGTCTTTTTATTTTGCTTTTGG		

Visualizing Amplified Products

Amplified DNA fragments were visualized and scored on 6% denaturing polyacrylamide gel and detected by silver staining. DNA fragments were scored for analysis.

Constructing phylogenetic tree, Image Analysis and Calculations

The unrooted phylogenetic tree was constructed using 1000 bootstrap values for the individuals using PHYLIP (Version 3.6) software. The observed and expected heterozygosity and effective number of alleles were calculated using 'MICROSAT' (version 1.5d) computer package (Version 1.5d). In addition, the genetic distances (D_A) were calculated according to Nei, 1983. Allele frequencies for each locus were calculated using diploid chromosome number (2n = 50) for each pig type.

RESULTS AND DISCUSSION

The application of molecular methods in evaluating the genetic diversity makes it possible to estimate the genetic variability between and within different populations and estimate the genetic distances and similarities among them. Nevertheless the molecular analysis could successfully be used to achieve the goals of conservation programs and to effectively orient the genetic improvement programs of native pigs.

Allelic distribution

Allele frequencies of 15 microsatellite loci for five pig populations (two native and three exotic types) were estimated using diploid chromosome number (2n=50). A total of 138 alleles were found, and among 15 microsatellite loci tested, 11 were polymorphic and the rest were (SW911, S0 1089, SW226 and SW335) monomorphic. 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 size 144 and 262 bp), SW218 (allele size 206 and 212 bp) and SW 226 (allele size 198 and 218 bp) were found to be unique. Wild boars carried two rear alleles (found only in few wild boars), namely locus SWR198 (allele size 122 bp) and locus SW 335 (allele size 98 bp). Village pigs carried only one rear allele (S0228, allele size 166 bp). However, no rear alleles were found among the three exotic breeds studied.

Heterozygosity

Table 2 shows the heterozygosity values and the mean number of alleles in five different swine populations. The genetic diversity within the population was assessed by effective number of alleles and heterozygosity (Cepica *et al.*, 1995). The mean effective number of alleles for 15 loci in wild boar, village pigs and exotic types (Landrace, Large White and Duroc) were 2 ± 0.38, 2 ± 0.53 and 1.73 ± 0.59, respectively (Table 2). The observed number of alleles and their frequencies indicated that the wild boar and village pigs have high allelic richness compared to exotic pig breeds.

The average values of observed heterozygosity are higher than the expected values of the 15 loci in all the populations (Table 2). The observed heterozygosity was higher in village

pigs (0.72 ± 0.02) compared to the other swine types. The higher heterozygosity may be due to the free range mating system, especially compared to the exotic breeds. Further, the lack of directional selection within the village pig population also might have contributed for it. Wild boar population showed the heterozygosity of 0.64 ± 0.02. The lower genetic diversity of exotic pigs indicated their purebred condition generally adopted in the process of developing commercial breeds.

Table 2. Heterozygosity values 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[a]
Village pigs	15	0.41±0.04	0.72±0.02	2.00±0.53[a]
Large White	15	0.34±0.06	0.53±0.05	1.73±0.59[b]
Land Race	15	0.34±0.07	0.56±0.05	1.73±0.59[b]
Duroc	15	0.33±0.06	0.53±0.05	1.73±0.59[b]

SD - Standard Deviation

The high heterozygosity values reflect high polymorphism exist within the population. The genetic diversity values in village pigs and wild boar recorded in the present study was lower than those reported in studies done in some other countries. For example, Desi and Guhuri pigs (0.77) in India (Behl *et al.*, 2002) and in Chineese (0.81) and Mexican pig populations (0.76) (Lemus-Flores *et al.*, 2001; Fang *et al.*, 2005). The higher genetic diversity levels present in Indian, Chinese and Mexican pig types may be the result of large effective population sizes exist in those countries compared to the size of village pigs and wild boar populations in Sri Lanka. Nevertheless, given the scattered distribution of village pig and wild boar populations in Sri Lanka, the comparatively low within population diversity may also be due to the isolated breeding in small populations limited to different geographical areas. However, 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).

Genetic Distance Within and Between Populations

Based on allelic frequency of 15 microsatellites loci, the genetic distances (D_A) between different swine populations were calculated according to the procedure described by Nei *et al*. (1983) and the results are shown in Table 3.

Table 3. Genetic distances (Nei *estimator*) estimated among 5 pig population

Population	1	2	3	4	5
1	-				
2	0.091	-			
3	0.262	0.261	-		
4	0.244	0.241	0.053	-	
5	0.284	0.268	0.040	0.040	-

1- Wild boar 2- Village pig 3-Large White 4- Landrace 5-Duroc

Based on allelic frequency of 15 microsatellites loci, the genetic distance (D_A) between village pigs and wild boars was found to be 0.091. The genetic distance between village pigs, Large White, Landrace and Duroc were found to be 0.261, 0.241 and 0.268, respectively, showing that the village pigs is a distinct population compared to exotic populations. This observation confirms the fact that the evolutionary path of village pig is completely separated from the evolutionary path of exotic pigs, whereas it is more close to the wild boars.

Phylogenetic Analysis

The unrooted phylogenetic tree was constructed using 1000 bootstrap values for the individuals in different swine populations. The phylogenetic consensus tree constructed using the neighbor joining method groups the individuals clearly into four distinct clusters. The clusters 1 and 3 consisted only with wild boars and village pigs, respectively. It shows that the village pig and wild boar populations genetically distinct and have diverged separately to certain extent. Interestingly, the cluster 2 consisted of both wild boar and village pigs. The low genetic distance (D_A) between two populations shown (Table 3) in genetic distance analysis above could be due to the admixture situation shown in the cluster 2. This leaves however, a question with regard to the origin and evolution of the village pig population of Sri Lanka. As it appears according to the present results, though the evolution of two pig population has taken place separately, there are different levels of introgression, especially in village pig population. This hypothesis needs to be investigated further by comparing the village pig and wild boar populations with other native populations in the region.

Most of the village pig samples collected from Chilaw and Puttalam were grouped in the cluster 3. In these areas pigs were kept under semi-intensive system of rearing (rearing in concrete pens and huts). This might limit mixing them with other wild pig populations. All the exotic pigs were clustered separately (cluster C4). The separate cluster of exotic pig population reveals the distinct genetic makeup of those from the native pig types.

The wild boar samples (WB6, WB9, WB12 and WB16) and some village pig samples (VP 20, VP22, VP 23 and VP 14) collected in Kurunegala were found in the cluster 2 (Fig. 2). Village pigs from Kurunegala district had long straight face, upwardly erect ears, angular body shape and long hairs densely found along the spine (Subalini *et al*., 2011). These traits are very similar to those of wild boars found 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 reserves where wild boars could be found. This agrees with the report by Nozawa (1980), who reported that in rural areas

where pigs are raised as free range, there is an opportunity for wild boars living in neighboring forests to mate with native pigs. The similar observation was reported for village pigs by Subalini *et al.* (2010) for Sri Lankan village pig rearing systems. 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 very similar body characteristics of wild boar. As in the case of Kurunegala area, in Kalutara district too the village pigs are managed under extensive system, which allows free crossing with other animals including wild boars. Accordingly, the gene flow from wild animals to domestic populations can frequently be observed in swine. The ability of the two populations to make free crossing and having fertile progeny is in support for the hypothesis of common origin of village pigs and wild boar. The similar situation was reported in a Chinese study by Zhao *et al.* (1990) and Zhang *et al.* (2003).

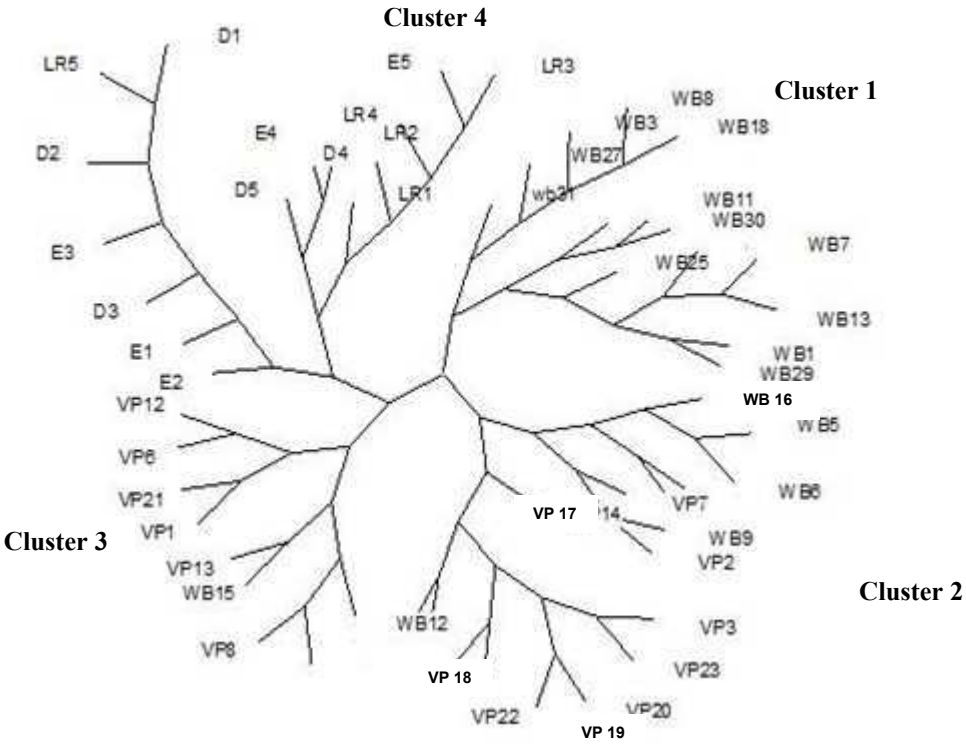

Fig. 2. The unrooted neighbour joining tree relating the allelic distribution of fifteen microsatellite markers among Wild Boar (WB) Village pigs (VP), Large White (L), Landrace (E) and Duroc (D)

The village pigs closely resemble the Sri Lankan wild boar in many aspects. The presence of horizontal stripes in newborn piglings of village pigs, which gradually disappear with age, is one of the proofs of the wild ancestry of village pigs. The tendency to use all four toes while standing and walking (Subalini *et al*., 2011) also links the village type to wild

boar, as it is characteristic of wild boar to use all four toes to get more footing in their natural habitat (Fisher and Devendra, 1963). The exotic breeds, in contrast, use only the two front toes. These observations supports the hypothesis that the village pigs in Sri Lanka might have evolved as a result of gradual domestication of wild boars. However, the phylogenetic analysis in the present study shows that there is a considerable genetic distance between the two populations, which could be due to diverse divergence path as mentioned earlier, though the two populations might have a common origin. Since the present study used manual scoring system in banding pattern, a thorough analysis with precise and objective tool is recommended before the confirmation of the hypothesis.

CONCLUSIONS

The findings of the study revealed that village pigs and wild boars are two genetically distinct populations. However, the village pigs in certain geographic areas of the country have 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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