



Individual identification and population assignment with microsatellite markers: an evaluation in two Indian pig populations

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ABSTRACT

Ability of a set of 24 microsatellite markers for individual identification and their potential for breed assignment of individuals was evaluated in 2 Indian pig populations. The cumulative probabilities of identity of 2 random individuals within a population, even with selected set of 5 loci (CGA, S0026, S0228, S0355, SW936) were 2.87×10^{-8} (Assamese) and 9.66×10^{-8} (Anakamali) and from 2 different population was 1.13×10^{-12} . However, the population assignment precision even with all the 24 loci was only 80 (Assamese) and 88% (Ankamali). These results suggested that although this set of markers can be safely employed for identification of individuals but their utility for breed allocation in Indian pigs needs further authentication before they can be practically used for such purposes.

Keywords: Breed assignment, Indian pigs, Individual identification

Owing to their highly polymorphic nature microsatellite DNA markers have been extensively used for analysis of phylogenetic relationships amongst populations in different species including pigs (Fang *et al.* 2005, Behl *et al.* 2006, SanCristobal *et al.* 2006, Sahoo *et al.* 2016, Gvozdanoviae *et al.* 2019, Ba *et al.* 2020). The utility of microsatellites have been also evaluated for parentage analysis in Chinese, European, Czech and Taiwanese pigs (Putnova *et al.* 2003, Fan *et al.* 2005, Lin *et al.* 2014, Yu *et al.* 2015). Similarly, some reports have suggested their utility in individual demarcation procedures like individual identification in Chinese pigs (Zhao *et al.* 2018) and assignment of an individual animal to a breed or population in Taiwanese, Spanish–French and Korean pigs (Kim *et al.* 2005, Boitard *et al.* 2010, Li *et al.* 2014). Although, recently we have evaluated their utility for parentage verification in Indian pigs (Behl *et al.* 2017), no such reports are available for individual identification and assignment to a breed or population in Indian pig populations. A test for the assignment of an individual to a breed is essential for effective and accurate selection and management of the livestock breeds. Besides, assignment of an individual animal to a population, the discrimination of individual animal is necessary for the authentication of the quality and origin of the livestock products. The present study was undertaken to evaluate a set of 24 microsatellite markers for their potential for individual identification and also to

assess their effectiveness in breed assignment of individual animals in 2 Indian pig populations.

MATERIALS AND METHODS

The blood samples were collected from 25 Assamese pigs from the state of Assam and 26 samples of Ankamali pigs from Kerala. The DNA was isolated by standard procedure of digestion with proteinase K, extraction with phenol/chloroform and precipitation with ethanol. The stock DNA was stored at -20°C and the working dilutions were stored at 4°C .

The genomic DNA was amplified by PCR using 24 microsatellite primers. Each 25 μl reaction consisted of DNA (approximately 100 ng), primers (60 ng), dNTPs (40 mM each), 10X buffer (10 mM tris, 50 mM KCl, 0.1% gelatin, pH 8.4) (2.5 μl), MgCl_2 (1.5 mM or as specified in FAO 1998) and Taq DNA polymerase (0.75 units). The thermo-cyclic conditions were initial denaturation at 92°C for 2 min followed by 30 cycles of denaturation at 94°C for 45s, annealing at the temperature given in FAO (1998) for 45s and extension at 72°C for 45s, with a final extension at 72°C for 10 min. The amplified fragments were analysed on 7% denaturing urea polyacrylamide gel and detected by silver staining (Bassam *et al.* 1991). The allele frequencies and within breed genetic diversity parameters of observed number of alleles (N_o) and observed heterozygosity (H_o) at each locus were calculated using POPGENE computer program version 1.31 (Yeh *et al.* 1999). The polymorphism information content (PIC) at each locus was calculated according to Botstein *et al.* (1980).

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The population allocation of individual animals was estimated by likelihood approach using frequency method (Paetkau *et al.* 1995) after 1000 simulations of the data with GENECLASS computer package (Piry and Cornuet 1998).

The probability of identity of 2 random individuals within a population (G1) or from 2 different populations (G2) was calculated as described by Van-Zeveren *et al.* (1995).

$$G_1 = \prod_{i=1}^r \left[\sum_{j=1}^{n_i} q_{ij}^4 + 4 \sum_{j=1}^{n_i} q_{ij}^2 - q_{ik}^2 \right]$$

with q_{ij} being the frequency of the j^{th} allele and i^{th} locus in a population.

$$G_2 = \prod_{i=1}^r \left[\sum_{j=1}^{n_i} q_{ij}^2 \cdot q'_{ij} + 4 \sum_{j=1}^{n_i} q_{ij} \cdot q'_{ij} \cdot q_{ik} \cdot q'_{ik} \right]$$

where, q and q' being the frequencies of corresponding alleles.

RESULTS AND DISCUSSION

The within breed genetic diversity parameters of observed and effective number of alleles, observed and expected heterozygosity at each locus in each population and genetic distances between these populations were published earlier (Behl *et al.* 2006). The probabilities of identity of 2 random individuals within a population (G1), taking into consideration all 24 loci were 1.54×10^{-33} and 7.28×10^{-36} in Assamese and Ankamali pigs, respectively (Table 1). The probability of identity of 2 random individuals from different populations (G2) with these 24 loci was 8.20×10^{-49} between these 2 pig populations (Table 1). The G2 values were clearly lower than the G1 values indicating that the probability of identity of 2 random individuals was clearly less between 2 individuals from different populations than from within a population. These values also showed the suitability of these loci to distinguish individual pigs or their products from 2 different populations or within a population.

To calculate the minimum number of loci required for developing a set of loci to achieve the specified minimum cumulative probabilities, the G1 and G2 values were calculated for a selected set of minimum of 5 loci then increasing the number of loci in increments of 5. The cumulative G1 values, even with the selected set of 5 loci (CGA, S0026, S0228, S0355, SW936) with $N_o > 9$, $H_o > 0.65$ and $PIC > 0.80$, were 2.87×10^{-8} in Assamese pigs and 9.66×10^{-8} in Anakamali pigs. The cumulative G2 between these 2 populations with this set of 5 loci was 1.13×10^{-12} . The cumulative G1 and G2 values with selected set of 10 loci (CGA, S0005, S0026, S0215, S0218, S0228, S0355, SW122, SW911, SW936), also meeting the above criterion for N_o , H_o and PIC , were 6.77×10^{-16} (Assamese) and 1.66×10^{-15} (Anakamali) for G1 and 3.92×10^{-24} for G2. These sets of 5 or 10 loci have sufficiently low probabilities

Table 1. Probability of identity of 2 random individuals from within a population (G1) and different populations (G2) in 2 Indian pig types of Assamese and Ankamali using a set of minimum 5 loci and then increasing the number of loci in increments of 5 up to maximum of 24 loci and 13 loci that were common with parentage verification kit for pigs recommended by ISAG

Number of loci employed	G1		G2
	Assamese	Ankamali	
5 (CGA, S0026, S0228, S0355, SW936)	2.87×10^{-8}	9.66×10^{-8}	1.13×10^{-12}
10 (Set of 5 loci and S0005, S0215, S0218, SW122, SW911)	6.77×10^{-16}	1.66×10^{-15}	3.92×10^{-24}
15 (Set of 10 loci and IGF1, S0068, S0090, S0155, S0178)	1.24×10^{-22}	1.17×10^{-22}	1.95×10^{-32}
20 (Set of 15 loci and S0225, S0226, S0227, S0386, SW24)	3.94×10^{-29}	1.09×10^{-29}	9.09×10^{-42}
All 24 (Set of 20 loci and SW72, SW632, SW857, SW951)	1.54×10^{-33}	7.28×10^{-36}	8.2×10^{-49}
13 (ISAG-S0005, S0090, S0155, S0227, S0228, S0355, S0386, SW124, SW72, SW857, SW911, SW936, SW951)	9.75×10^{-18}	4.41×10^{-19}	1.71×10^{-26}

of identities that they can be safely employed for the individual identification procedures in Indian pig populations. The cumulative G1 and G2 values with the 13 loci that were common with the set recommended by International Society for Animal Genetics (ISAG) were 9.75×10^{-18} (Assamese) and 4.11×10^{-19} (Anakamali) for G1 and 1.71×10^{-26} for G2. The gradually decreasing match probability values with the increasing number of loci indicated an enhanced traceability accuracy. Moreover, in the recent times, the DNA genotyping has become most cost effective method for pedigree maintenance in large populations of animals because of the drastic decrease in the price of genotyping. The microsatellites have an added advantage of multiplexing over SNP based methods thus saving time and energy. Moreover, the SNPs are generally biallelic compared to highly polymorphic nature of microsatellites.

Besides distinguishing between individuals in breeding and conservation programmes, the allocation of an individual to a population is equally important to discriminate between purebreds and crossbreds for skilful management of the animal genetic resources. If a method could be developed for authentication of breed or population of an individual it could be of great help to the breeders. Although, the possibilities of using microsatellites for assigning breed identities to anonymous samples have been evaluated in Taiwanese, Spanish-French and Korean pig

populations (Kim *et al.* 2005, Boitard *et al.* 2010, Li *et al.* 2014), no such reports are available in Indian pigs. We attempted to evaluate the potential of the above set of 24 microsatellite loci for population assignment in Indian pig populations by likelihood approach using frequency method (Paetkau *et al.* 1995).

Only those animals were considered to be unambiguously assigned to a population that had an assignment probability to that population to be clearly higher than that of the second most probable population. If the ratio of the most likely allocation with the second most likely allocation approaches one, it is assumed that there is ambiguity in the assignment of the particular animal (Banks and Eichert 2000). In the likelihood approach, a population is almost always designated because there is always a most likely or a closest population in a reference set. In practical conditions the animal to be assigned may not belong to any of the populations under consideration (Cornuet *et al.* 1999). Therefore, a minimum assignment probability of 0.05 was adopted for unambiguous assignment of an individual to a population. With this stringency, with selected set of 5 loci (CGA, S0026, S0228, S0355, SW936) only 72.0 (Assamese) to 76.9% (Ankamali) correct assignments were achieved (Table 2). Similarly, with the selected set of 10 loci (CGA, S0005, S0026, S0215, S0218, S0228, S0355, SW122, SW911, SW936) only 76.0 (Assamese) to 80.8% (Ankamali) correct assignments were achieved. Several factors have been proposed to affect the accuracy of such individual specific demarcation procedures such as genetic differentiation between the populations in question and degree of reproductive isolation etc. (Cornuet *et al.* 1999). Although, these two pig populations are geographically isolated with Nei's D_A genetic distance of 0.246 (Behl *et al.* 2006). However, even with all the 24 loci, only 80.0 (Assamese) to 88.0% (Ankamali) correct assignments were achieved. The results obtained in our study are in disagreement to the other studies in Taiwanese, Spanish, French and Korean pig populations, in which the potential of using microsatellite loci for assigning breed identities to

Table 2. Per cent of unambiguously assigned animals of 2 Indian pig populations after allocation with frequency based likelihood method of Paetkau *et al.* (1995) with method after 1000 simulations of the data using using a set of minimum 5 loci and then increasing the number of loci in increments of 5 up to maximum of 24 loci and 13 loci that were common with parentage verification kit for pigs recommended by ISAG

Number of loci employed*	Per cent unambiguous assignments	
	Assamese	Ankamali
5	72.0	76.9
10	76.0	80.8
15	84.0	84.6
20	76.0	88.0
All 24	80.0	88.0
13 (ISAG)	84.0	92.0

*See Table 1 for loci names.

anonymous pig samples has been proposed (Kim *et al.* 2005, Boitard *et al.* 2010, Li *et al.* 2014). One possible reason for comparatively lower assignment precision in our study could be that the Indian pig populations/breeds may not be as well differentiated as purebred stock of the Western or Oriental pig breeds.

The above results suggested that although, even the set of 5 or 10 microsatellite loci showed sufficiently low probabilities of identities indicating their suitability for individual identification purposes in Indian pigs. However, their usefulness for individual assignment to a breed or a population may perhaps require further substantiation before they can be employed in Indian pigs.

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