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

RAHUL BEHL1,2, JYOTSNA DHINGRA BEHL1, N NAHARDEKA1, G C DAS1, K SAJEEV KUMAR1, K ANIL KUMAR1, M S TANTIA1 and R K VIJH1

ICAR-National Bureau of Animal Genetic Resources, Karnal, Haryana 132 001 India

Received: 22 February 2021; Accepted: 30 July 2021

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, Gvozdanovic 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), MgCl₂ (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) 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).
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).

\[ G_i = \prod_{i=1}^{r} \left\{ \sum_{j=1}^{n_i} q_{ij}^i + 4 \sum q_{ij}^i - q_{ik}^i k \right\} \]

\[ G_r = \prod_{i=1}^{r} \left\{ \sum_{j=1}^{n_i} q_{ij}^r - q_{ijk} q_{ik} \right\} \]

where, \( q_i \) being the frequency of the \( j \)th allele and \( i \)th locus in a population.

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 up to maximum of 24 loci and 13 loci that were common with parentage verification kit for pigs recommended by ISAG

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

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 skillful 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 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

<table>
<thead>
<tr>
<th>Number of loci employed*</th>
<th>Per cent unambiguous assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Assamese</td>
</tr>
<tr>
<td>5</td>
<td>72.0</td>
</tr>
<tr>
<td>10</td>
<td>76.0</td>
</tr>
<tr>
<td>15</td>
<td>84.0</td>
</tr>
<tr>
<td>20</td>
<td>76.0</td>
</tr>
<tr>
<td>All 24</td>
<td>80.0</td>
</tr>
<tr>
<td>13 (ISAG)</td>
<td>84.0</td>
</tr>
</tbody>
</table>

*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.

REFERENCES


Gvozdanoviæ K, Margeta V, Margeta P, Kušec I D, Galoviæ D, Đove&and Wkud Kušec G. 2019. Genetic diversity of autochthonous pig breeds analyzed by microsatellite markers and