Evaluation of the genetic variability of 13 microsatellite markers in native Indian pigs

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Abstract

We analysed polymorphism of 13 microsatellites in two Indian domesticated pig types (North Indian and Northeast Indian). Heterozygosity, polymorphism information content, and probability of identity of two random individuals were calculated for all microsatellites in both types. The number of alleles observed at a locus varied between five and 12. The evaluated microsatellites exhibited a very high heterozygosity and polymorphism information content. The probability of identity of two random individuals from different populations taking into account all the 13 microsatellites was as low as 3.51×10^{-19}. On the basis of these results, we propose that these microsatellite markers may be used with reliability for studying the genetic diversity and for identification of individuals in Indian pig types.

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Introduction

In the world of animal production, a good method to identify animals and their products unambiguously is of utmost importance in obtaining accurate selection and high-quality products. In light of continuously growing demand for better products, faster and more reliable methods of identification of individuals are essential. Identification methods such as typing of blood groups and biochemical polymorphism have proved their usefulness, but the discriminating power of these techniques is less than that of DNA markers. Moreover, the number of different tissues on which the typing can be done is very limited and represents a significant limitation of such methods. Several DNA-based technologies to type polymorphic loci have been developed in the last decade. The techniques include restriction fragment length polymorphism, variable number of tandem repeats, single strand conformational polymorphism, denaturing gradient gel electrophoresis, random amplified polymorphic DNA, and also methods that make use of polymorphism of short tandem repeats, i.e. microsatellites. There are now more than a thousand pig microsatellites to choose from (Archibald *et al*. 1995). The Food and Agriculture Organization of the United Nations (FAO) has recommended a set of microsatellites for evaluating the genetic diversities of pigs (Food and Agriculture Organization 1998). Use of the same set of markers will produce comparable results (Hammond and Leitch 1998). These microsatellite markers have been evaluated and used for genetic variability studies in various European and Chinese pig breeds (Van-Zeveran *et al*. 1995; Laval *et al*. 2000; Li *et al.* 2000; Martinez *et al*. 2000). The aim of the present study was to evaluate 13 microsatellite loci for their utility in identification of individuals in two native domesticated pig types of India.

Materials and methods

***Samples*:** A total of 50 blood samples (25 samples per pig type) were collected from genetically unrelated pigs from the states of Haryana, representing the North Indian type, and Assam, representing the Northeast Indian type. Blood was collected aseptically into Vacutainers (Becton and Dickinson, USA) containing heparin from the anterior vena cava by holding the animal in dorsal reincumbency.

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DNA was isolated by cell lysis and extracted with phenol/chloroform. The DNA samples were stored at – 20°C and/or at 4°C.

***Polymerase chain reaction*:** Genomic DNA was amplified by polymerase chain reaction using the FAO recommended microsatellite primers (table 1). Primers were synthesized by Gemini Biotech, USA. Each 25-µl reaction consisted of DNA (100 ng), primers (60 ng each), dNTPs (Promega, USA; 40 mM each), 2.5 µl of 10 × buffer (10 mM Tris, 50 mM KCl, 0.1% gelatin; pH 8.4), $MgCl_2$ (1.5 mM or as mentioned in table 1) and *Taq* DNA polymerase (Promega; 0.75 unit). The thermocycle conditions were: 2 min at 94°C followed by 30 cycles of 45 s at 94°C, 45 s at annealing temperature (table 1) and 45 s at 72°C. The last elongation step was prolonged to 10 min. The reaction was stopped by soaking the tubes at 4°C. Amplified DNA fragments were analysed on 8% denaturing polyacrylamide gel and detected by silver staining (Yang *et al*. 1999). Fragment size was calculated according to Seally and Southern (1990).

***Statistical analysis*:** Allele frequencies for each locus were calculated with $2n = 50$ for each pig type. The heterozygosity or genetic diversity was calculated according to Nei (1978) using the POPGENE computer package (Yeh *et al*. 1999). Polymorphism information content (PIC) values were calculated by using the method

Table 1. Microsatellite loci and their chromosomal locations, primer sequences, and the PCR conditions used.

Microsatellite locus	Chromosome location	Primer sequence	Annealing temp. (°C)	$MgCl_2$ conc. (µM)
CGA	1	ATAGACATTATGTCCGTTGCTGAG GAACTTTCACATCCCTAAGGTCGT	62	1.5
S0005	5	TCCTTCCCTCCTGGTAACTA GCACTTCCTGATTCTGGGTA	58	1.5
20068	13	AGTGGTCTCTCTCCCTCTTGCT CCTTCAACCTTTGAGCAAGAAC	62	1.5
S090	12	CCAAGACTGCCTTGTAGGTGAATA GCTATCAAGTATTGTACCATTAGG	58	1.5
S0218	10	GTGTAGGCCTGGCGGTTGT CCCTGAAACCTAAAGCAAAG	55	2.0
S0225	8	GCTAATGCCAGAGAAATGCAGA CAGGTGGAAAGAATGGAATGAA	55	4.0
S0226	2	GCACTTTTAACTTTCATGATACTCC GGTTAAACTTTTNCCCCAATAGA	55	4.0
S0227	4	GATCCATTTATAATTTTAGCACAAAGT GCATGGTGTGATGCTATGTCAAGC	55	4.0
S0335	15	TCTGGCTCCTACACTCCTTCTTGATG TTGGGTGGGTGCTGAAAAATAGGA	55	4.0
SW24	17	CTTTGGGTGGAGTGTGTGC ATCCAAATGCTGCAAGCG	58	1.5
SW72	3	ATCAGAACAGTGCGCCGT TTTGAAAATGGGGTGTTTCC	58	1.5
SW122	6	TTGTCTTTTTATTTTGCTTTTGG CAAAAAAAGGCAAAAGATTGACA	58	1.5
SW911	9	CTCAGTTCTTTGGGACTGAACC CATCTGTGGAAAAAAAAAGCC	60	1.5

described by Botstein *et al*. (1980). The probability of identity of two individuals chosen at random in a population is given by:

$$G1 = \prod_{i=1}^{r} \left[\sum_{j=1}^{n_i} q_{ij}^4 + 4\Sigma\Sigma q_{ij}^1 \cdot q_{ik}^2 \right],$$

with q_{ij} being the frequency of the jth allele at the ith locus in a population.

The probability of identity of two individuals belonging to two different types was calculated as follows:

$$G2 = \prod_{i=1}^{r} \left[\sum_{j=1}^{n_i} q_{ij}^2 q_{ij}'^2 + 4\Sigma\Sigma q_{ij} \cdot q_{ij}' \cdot q_{ik} \cdot q_{ik}' \right],$$

with q and q' being the frequencies of the corresponding alleles.

Results and discussion

Allele frequencies for the 13 microsatellites in the two populations are presented in table 2. The number of alleles, heterozygosity, PIC and probability of genetic identity at each marker of two individuals from the same population and from the two populations ($G1$ and $G2$) are given in table 3. The number of alleles detected ranged from five (at three loci) to 12 (locus S0355 in North Indian pigs). The mean observed and expected heterozygosities were 0.68 ± 0.13 and 0.81 ± 0.05 in the North Indian and 0.66 ± 0.12 and 0.83 ± 0.04 in the Northeast Indian pig types, respectively.

The average observed heterozygosities are lower than the expected values in both the populations. Though the heterozygosities observed in the studied Indian pig populations is similar to the values reported for microsatellites in Chinese pig breeds (Li *et al*. 2000a,b), they are a little higher than the values reported for European pig breeds

Table 2. Numbers and sizes of alleles (in bp) and allele frequencies at 13 microsatellite loci in North Indian (NR) and Northeast Indian (NE) pigs.

Locus and allele size	NR	NE	Locus and allele size	NR	NE	Locus and allele size	NR	NE	Locus and allele size	NR	NE
CGA			**S0090**			**S0027**			**SW72**		
266	0.06	0.04	243	0.12	0.12	231	0.02	0.00	100	0.04	0.00
270	0.00	0.14	245	0.20	0.32	233	0.00	0.02	104	0.10	0.00
274	0.04	0.04	247	0.04	0.26	237	0.08	0.16	106	0.00	0.04
278	0.14	0.14	249	0.32	0.18	239	0.42	0.42	108	0.34	0.08
282	0.22	0.14	251	0.32	0.12	241	0.12	0.16	110	0.30	0.34
284	0.26	0.16	**S0218**			243	0.08	0.00	112	0.22	0.20
288	0.08	0.12	164	0.06	0.00	245	0.04	0.16	114	0.00	0.20
292	0.04	0.04	168	0.08	0.06	247	0.12	0.00	116	0.00	0.14
294	0.02	0.08	172	0.30	0.08	249	0.10	0.04	**SW122**		
298	0.08	0.04	174	0.20	0.26	253	0.02	0.04	110	0.06	0.06
302	0.06	0.06	176	0.22	0.34	**S0355**			112	0.00	0.04
S0005			178	0.00	0.20	247	0.02	0.02	114	0.16	0.04
215	0.00	0.16	180	0.08	0.04	249	0.10	0.00	116	0.32	0.12
221	0.08	0.16	182	0.02	0.00	251	0.02	0.08	118	0.24	0.26
231	0.04	0.00	184	0.04	0.02	255	0.04	0.14	120	0.14	0.20
233	0.04	0.08	**S0225**			257	0.20	0.04	122	0.06	0.18
235	0.08	0.08	172	0.00	0.04	259	0.14	0.20	124	0.02	0.06
237	0.12	0.04	176	0.00	0.12	261	0.28	0.30	128	0.00	0.02
239	0.22	0.22	178	0.16	0.16	263	0.06	0.12	132	0.00	0.02
241	0.08	0.14	182	0.20	0.18	265	0.02	0.00	**SW911**		
243	0.06	0.04	184	0.00	0.06	267	0.04	0.04	153	0.00	0.04
245	0.18	0.08	186	0.34	0.30	269	0.02	0.02	157	0.08	0.10
251	0.06	0.00	190	0.26	0.10	273	0.06	0.04	159	0.04	0.00
257	0.04	0.00	194	0.04	0.04	**SW24**			161	0.16	0.08
S0068			**S0226**			92	0.02	0.06	165	0.20	0.24
218	0.06	0.06	185	0.02	0.00	96	0.14	0.18	167	0.16	0.12
222	0.02	0.16	187	0.04	0.00	100	0.36	0.26	169	0.24	0.30
224	0.18	0.04	189	0.10	0.06	104	0.10	0.06	171	0.08	0.04
226	0.16	0.16	191	0.18	0.14	108	0.30	0.26	175	0.04	0.08
228	0.18	0.22	193	0.20	0.28	112	0.08	0.18			
230	0.10	0.12	197	0.08	0.18						
234	0.18	0.14	201	0.34	0.28						
238	0.10	0.06	205	0.04	0.06						
242	0.02	0.04									

Table 3. Observed numbers of alleles, PCR product size range, observed and expected heterozygosity, polymorphism information content (PIC), and probability of identity of two individuals chosen at random from within a group (*G*1) and from the two different groups (*G*2) at 13 microsatellite loci in two native pig types of India.

	North Indian pigs						Northeast Indian pigs						
Micro-satellite locus	Observed no. of alleles	Size range (bp)	Observed hetero-zygosity	Expected hetero-zygosity	PIC	*G*1	Observed no. of alleles	Size range (bp)	Observed hetero-zygosity	Expected hetero-zygosity	PIC	*G*1	*G*2
CGA	10	266–302	0.76	0.85	0.83	0.049	11	266–302	0.84	0.90	0.88	0.024	0.023
S0005	11	221–257	0.84	0.89	0.87	0.028	9	215–245	0.64	0.87	0.86	0.036	0.015
S0068	9	218–242	0.64	0.87	0.84	0.039	9	218–242	0.68	0.87	0.85	0.037	0.027
S0090	5	243–251	0.76	0.75	0.74	0.113	5	243–251	0.72	0.78	0.75	0.092	0.079
S0218	8	164–184	0.68	0.81	0.78	0.066	7	168–184	0.60	0.78	0.74	0.091	0.042
S0225	5	178–194	0.48	0.76	0.72	0.105	8	172–194	0.60	0.83	0.80	0.069	0.060
S0226	8	185–205	0.64	0.80	0.78	0.091	6	189–205	0.52	0.80	0.70	0.115	0.061
S0227	9	231–253	0.64	0.78	0.75	0.075	7	233–253	0.48	0.75	0.72	0.099	0.065
S0355	12	247–273	0.84	0.85	0.82	0.047	10	247–273	0.60	0.84	0.81	0.052	0.031
SW24	6	92–112	0.72	0.76	0.73	0.064	6	92–112	0.76	0.80	0.78	0.074	0.035
SW72	5	100–112	0.52	0.75	0.70	0.117	6	106–116	0.68	0.79	0.76	0.083	0.047
SW122	7	110–124	0.48	0.80	0.77	0.076	10	110–132	0.68	0.85	0.82	0.047	0.039
SW911	8	157–175	0.84	0.85	0.87	0.048	8	153–175	0.88	0.82	0.81	0.059	0.024

(Fredholm *et al*. 1993; Van-Zeveran *et al*. 1995; Laval *et al*. 2000). The lower heterozygosity values for European breeds, compared to Indian breeds, may be because European breeds are highly specialized and are represented by smaller numbers of animals. Heterozygosity reflects the genetic diversity of a population and thus, to a certain extent, also reflects the state of inbreeding (Cepica *et al*. 1995). The mean numbers of alleles for North Indian and Northeast Indian pig types were 7.92 ± 2.29 and 7.84 ± 1.86 respectively. A high proportion (about 0.7 to 0.8) of the population was expected to be heterozygous for these markers, the expected heterozygosity being a function of the number of alleles and their frequencies.

The PIC values ranged between 0.72 (S0225) and 0.87 (SW911) in North Indian pigs and between 0.70 (S0226) and 0.88 (CGA) in Northeast Indian pigs. These numbers suggest suitability of these 13 microsatellites as markers of choice for genetic distancing studies of Indian pig. The probability of genotypic identity at all 13 loci for two randomly picked individuals from within the same group is 3.67×10^{-17} for the North Indian type and 1.86×10^{-16} for the Northeast Indian type. The probability of genotypic identity of two randomly picked individuals from the two groups was found to be 3.5×10^{-19} taking into account all the 13 microsatellites studied. Van–Zeveran *et al*. (1995), on the basis of their studies in Belgian pigs, have shown that the genetic diversity calculated on the basis of microsatellite polymorphism is always higher than that based on protein polymorphisms and that the former values are more accurate. It seems that the high mutation rate of microsatellites, estimated to be 4.5×10^{-4} in case of pigs (Li *et al*. 2000b), makes these short tandem repeats well suited for evolutionary studies of closely related breeds and species as well as for identifying individuals.

In conclusion, the panel of microsatellites evaluated in native pigs of India in the present study showed a very high heterozygosity and polymorphism and very low probability of genetic identity of two individuals belonging to two different populations. We propose that this set of microsatellites may be used for identifying individuals and for genetic diversity studies for selection and conservation of Indian pigs.

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