Molecular characterization of Kenkatha and Gaolao (*Bos indicus*) cattle breeds using microsatellite markers

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Abstract

*Molecular characterization of Kenkatha and Gaolao (*Bos indicus*) cattle breeds using microsatellite markers.*—One hundred forty–five individuals from two cattle breeds, Kenkatha and Gaolao, in India were studied using 25 fluorescently–labelled microsatellite markers. Genetic diversities within and between populations were studied. A total of 197 and 239 distinct alleles were identified across 25 microsatellite loci in Kenkatha and Gaolao cattle, respectively. Means of observed and expected heterozygosity were found to be 0.47 ± 0.24 and 0.62 ± 0.21 in Kenkatha, and 0.53 ± 0.17 and 0.68 ± 0.14 in Gaolao cattle, respectively. The average PIC (Polymorphic Information Content) value was found to be 0.59 ± 0.21 for Kenkatha and 0.65 ± 0.15 for Gaolao cattle. The mean fixation index (F_{IS}) was 0.2121 for Gaolao and 0.2248 for Kenkatha cattle. Mean F_{IS}, mean F_{IT} and mean F_{ST} (*F*–statistics) values were found to be 0.2318, 0.2487 and 0.0219, respectively. Nei's standard genetic distance value between Kenkatha and Gaolao breeds was 0.0852. The present study indicates that there is a substantial shortfall, 21.21% and 22.48%, of heterozygotes in Gaolao and Kenkatha cattle populations, respectively; and little genetic differentiation (2.19%) between the two breeds.

Key words: Kenkatha cattle, Gaolao cattle, Microsatellite markers.

Resumen

*Caracterización de las razas Kenkatha y Gaolao del cebú (*Bos indicus*) utilizando marcadores microsatélites.*—Se estudiaron 145 individuos de dos razas de cebús en la India, Kenkatha y Gaolao, utilizando 25 microsatélites marcados por fluorescencia. Se estudiaron las diversidades genéticas dentro y entre poblaciones. Se identificaron un total de 197 y 239 alelos distintos de entre 25 loci de microsatélites en los cebús Kenkatha y Gaolao, respectivamente. Se halló que las medias de la heterocigosidad observada y esperada eran de 0,47 ± 0,24 y 0,62 ± 0,21 en la raza Kenkatha y de 0,53 ± 0,17 y 0,68 ± 0,14 en la raza Gaolao, respectivamente. El valor de PIC (Contenido de Información Polimórfica) hallado fue de 0,59 ± 0,21 para Kenkatha y 0,65 ± 0,15 para Gaolao. El índice de fijación (F_{IS}) medio fue de 0,2121 para Gaolao y de 0,2248 para Kenkatha. Se vio que los valores del F_{IS} medio, el F_{IT} medio y el F_{ST} medio (distribución *F*) eran de 0,2318, 0,2487 y 0,0219, respectivamente. El valor de la distancia genética estándar de Nei entre las razas de Kenkatha y Gaolao fue de 0,0852. El presente estudio indica que existe un considerable déficit, del 21,21% y el 22,48%, de heterocigotos en las poblaciones de cebú Gaolao y Kenkatha, respectivamente; además de una diferenciación genética escasa (2,19%) entre ambas razas.

Palabras clave: Cebú Kenkatha, Cebú Gaolao, Marcadores microsatélites.

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Introduction

Indian cattle, also known as zebu cattle (*Bos indicus*), are broadly categorized into dairy, dual and draught purpose breeds depending on their utility. In India, there are 30 documented breeds of zebu cattle besides numerous populations in various states of India that are yet to be characterized and defined (Nivsarkar et al., 2000). Gaolao is a dual purpose breed found in the Wardha district of Maharashtra State, and Balaghat, Chhindwara and Seoni districts of Madhya Pradesh State of India. The Kenkatha breed of cattle, also known as Kenwariya, got the name from the River Ken as they are bred along the banks of this small river in the Bundelkhand region of Madhya Pradesh (Panna, Chhatarpur and Tikamgarh districts) and adjoining Hamirpur district of Uttar Pradesh State. Bullocks of this breed are very popular for light draught on the road and for cultivation. In the past, although farmers maintained a large number of Kenkatha animals in their breeding tract only 16,947 heads of this breed are recorded. This may be due to unrestricted interbreeding of Kenkatha with non–descript animals. As a result, the breed is becoming diluted and facing degeneration (Tomar et al., 2008). Immediate steps to conserve and improve this breed are therefore warranted.

Microsatellite markers are considered a marker of choice to characterize breeds for diversity assessment (FAO, 2007). Their short length makes them amenable to amplification by polymerase chain reaction (Weber & May, 1989; Wang et al., 1998). Microsatellites have been effectively exploited to evaluate genetic diversity and relationships among cattle populations (Ashwell et al., 2004; Sun et al., 2007). Microsatellite analysis using fluorescently–labelled primers and capillary fractionation is the pre–eminent method for the genetic analysis of eukaryotic organisms (Fatima, 2007). Information regarding phenotypic characterization of both breeds is available but molecular characterization using fluorescently–labeled microsatellite markers is lacking. The aim of the present study was to characterize Gaolao and Kenkatha breeds cattle at a molecular level by means of analysis of within and between breeds genetic variability of 25 fluorescently labelled microsatellite markers.

Materials and methods

Sample collection and DNA extraction

Blood samples from 145 purebred, randomly selected, unrelated cattle (70 Kenkatha and 75 Gaolao) were collected from various villages in their respective breeding region (Panna, Chhatarpur and Tikamgarh districts in Madhya Pradesh State for Kenkatha; Wardha district of Maharashtra State, and Balaghat, Chhindwara and Seoni districts in Madhya Pradesh State for Gaolao cattle). Genomic DNA was extracted using the method developed by John et al. (1991).

Microsatellite genotyping

Twenty–five microsatellite markers were selected from the database (http://www.fao.org/dad_is) recommended by the Food and Agriculture Organisation and the International Society for Animal Genetics (FAO and ISAG), and suggested by NBAGR (National Bureau of Animal Genetic Resources, Karnal, India, 2003). The forward primer of each pair was labelled with one of the four fluorophores, i.e. FAM, HEX, TET or ROX dye phosphoramidites which were synthesized by Applied Biosystems, USA. All 25 microsatellite markers were arranged by fragment size and fluorescent dye label into 4 PCR multiplexed panels carrying 10, 6, 5 and 4 markers per panel. PCR (Polymerase Chain Reaction) amplifications were performed on a thermal cycler (Eppendorf) in 15 µl reaction using 7.5 µl (1X) 2X PCR Hotstart Mastermix (Qiagen), primer mix of reverse 2.0 µl and forward 2.0 µl (2.0 pmol each), 2.0 µl DNA template (60 ng) and DNAase free water (1.5 µl) to make a final reaction volume of 15 µl. Each panel was run in one gel lane on an ABI–310® genetic analyzer (Applied Biosystem, USA). Microsatellite fragment sizing was performed using software Gene Mapper™ version 3.7 (Applied Biosystems, USA). Allele calling was performed with the software and was also checked manually to avoid any false calling of alleles.

Statistical analysis

Different measures of within–breed genetic variations, namely observed number of alleles (*no*), effective number of alleles (*ne*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), and the within–population inbreeding estimate also known as Wright's (1978) fixation index (F_{IS}) at each microsatellite locus were estimated to evaluate variability at DNA level using the POPGENE software package (Yeh et al.,1999). Polymorphic information content (PIC) for each locus was calculated according to Botstein et al. (1980). Departure from Hardy–Weinberg proportions was determined using exact probability tests provided in GENEPOP version 3.1 a (Raymond & Rousset, 1995). *F*–Statistics to describe the properties of a subdivided population, and Nei's measures of genetic identity and distance (Nei, 1972) were estimated using the POPGENE software package (Yeh et al., 1999).

Results

All 25 microsatellites in both Gaolao and Kenkatha cattle were successfully amplified in four multiplexes. Across 25 microsatellite loci studied, a total of 239 and 197 distinct alleles were observed in Gaolao and Kenkatha cattle, respectively. In Gaolao cattle 14 of 239 alleles were private alleles (locus ETH10, ETH152, HEL51, ILSTS005, ILSTS006, ILSTS0554, INRA005, MM8, HAUT24) while in Kenkatha cattle 6 of 197 alleles were private alleles (locus CSRM60, ETH185, HAUT27, INRA063). These private alleles can be used to differentiate the

Table 1. Allele size range (bp) observed, number of alleles (*no*, observed; *ne*, effective) and heterozygosity (*Ho*, observed; *He*, expected) for 25 microsatellite loci in Gaolao and Kenkatha cattle: * Non–significant for HWE (Hardy–Weinberg equilibrium).

*Tabla 1. Rango del tamaño de los alelos (pb) observado, número de alelos (*no*, observados;* ne*, efectivos) y heterocigosidad (*Ho*, observada;* He*, esperada) para 25 loci de microsatélites en los cebús Gaolao y Kenkatha: * No significativo para el equilibrio de Hardy–Weinberg (EHW).*

			Number of alleles				Heterozygosity			
	Allele size range		Gaolao		Kenkatha		Gaolao		Kenkatha	
Locus	Gaolao	Kenkatha	*no*	*ne*	*no*	*ne*	*Ho*	*He*	*Ho*	*He*
BM1818	258–280	258–274	11	5.96	9	4.74	0.6379	0.8396	0.7246	0.7952
BM1824	176–190	176–186	6	2.11	4*	1.43	0.6806	0.5317	0.3571	0.3064
CSRM60	86–118	86–120	10	1.97	9*	2.63	0.3733	0.4967	0.9143	0.6243
CSSM663	174–204	172–206	14	3.58	9	3.00	0.3378	0.7261	0.4706	0.6717
ETH3	103–121	103–121	8*	2.05	6	1.17	0.5200	0.5162	0.0725	0.1523
ETH10	203–223	205–221	11	3.62	9*	3.86	0.7027	0.7288	0.7714	0.7469
ETH152	188–204	188–204	8	1.49	7	1.41	0.2286	0.3330	0.2537	0.2953
ETH185	221–247	209–247	12	8.86	13	6.17	0.6515	0.8939	0.1875	0.8447
HAUT24	168–270	180–284	13	5.15	10	3.98	0.5270	0.8114	0.3824	0.7545
HAUT27	132–148	130–148	8	4.73	10	6.13	0.4118	0.7946	0.4894	0.8460
HEL001	97–119	101–119	9	2.87	9	3.13	0.4521	0.6573	0.3971	0.6859
HEL009	132–166	132–166	12	6.90	11	5.36	0.7432	0.8610	0.7206	0.8195
HEL51	146–170	146–168	11	2.37	6	1.35	0.3649	0.5836	0.2464	0.2622
ILSTS005	176–190	176–186	7	1.96	5*	1.26	0.5833	0.4956	0.2143	0.2124
ILSTS006	279–301	279–297	5	2.03	4	2.16	0.1000	0.5116	0.0141	0.5415
ILSTS011	260–272	262–272	7	2.27	6	3.02	0.4028	0.5653	0.3286	0.6741
ILSTS030	147–155	147–155	5*	3.26	5	2.67	0.7500	0.6981	0.50	0.6301
ILSTS033	132–146	134–152	8	3.59	7*	3.12	0.6933	0.7269	0.6857	0.6846
ILSTS034	138–170	126–168	15	9.66	14*	8.43	0.6761	0.9029	0.78	0.8881
ILSTS0554	133–155	143–153	9	3.58	6	2.60	0.6933	0.7262	0.5143	0.6207
INRA005	132–144	132–140	6*	3.02	5	3.57	0.6486	0.6742	0.5645	0.7259
INRA035	96–118	96–118	12	4.67	8	5.10	0.5135	0.7913	0.3286	0.8101
INRA063	176–188	170–186	7	3.72	6*	2.39	0.4267	0.7363	0.7571	0.5871
MM8	118–150	120–148	12	4.48	10	3.61	0.7361	0.7822	0.7206	0.7290
MM12	94–120	96–120	12	5.85	9	5.97	0.5205	0.8349	0.3676	0.8387
Mean			9.52	3.99	7.92	3.53	0.5350	0.6888	0.47	0.62
SD			2.84	2.12	2.61	1.85	0.1730	0.1485	0.24	0.21

two breeds. The mean numbers of alleles observed were 9.52 for Gaolao and 7.92 for Kenkatha cattle (table 1). Alleles observed per locus ranged between 5 (loci ILSTS006 and ILSTS030) and 15 (locus ILSTS034) in Gaolao cattle and between 4 (locus ILSTS006) and 14 (loci BM1824 and ILSTS006) in Kenkatha cattle (table 1).

The observed heterozygosity (*Ho*) ranged between 0.0141 (ILSTS006) and 0.7800 (ILSTS034) in Kenkatha, and between 0.1000 (ILSTS006) and 0.7500 (ILSTS030) in Gaolao cattle (table 1). Expected heterozygosity (*He*) ranged between 0.1523 (ETH3) and 0.8881 (ILSTS034) in Kenkatha, and between 0.3330 (ETH152) and 0.9029 (ILSIS034) in

Table 2. Polymorphic Informtion Content (PIC) values, F_{IS} values and *F*–Statistics analysis for 25 microsatellite loci in Gaolao and Kenkatha cattle: * Wright's (1978) fixation Index.

Tabla 2. Valores de contenido de información polimórfica (PIC), valores F_{IS}*, y distribución* F *para 25 loci de microsatélites en los cebús Gaolao y Kenkatha: * Índice de fijación de Wright (1978).*

	PIC		*F_{IS}		*F*–Statistics		
Locus	Gaolao	Kenkatha	Gaolao	Kenkatha	F_{IS}	F_{IT}	F_{ST}
BM1818	0.81	0.77	0.2336	0.0821	0.1598	0.1661	0.0075
BM1824	0.48	0.28	–0.2890	–0.1741	–0.2470	–0.2161	0.0248
CSRM60	0.48	0.57	0.2434	–0.4751	–0.1567	0.0486	0.1774
CSSM663	0.69	0.61	0.5315	0.2942	0.4175	0.4317	0.0243
ETH3	0.50	0.15	–0.0140	0.5208	0.1078	0.1503	0.0477
ETH10	0.68	0.70	0.0293	–0.0403	–0.0059	–0.0043	0.0016
ETH152	0.32	0.28	0.3086	0.1342	0.2267	0.2299	0.0042
ETH185	0.88	0.82	0.2656	0.7763	0.5137	0.5243	0.0218
HAUT24	0.79	0.71	0.3460	0.4895	0.4151	0.4365	0.0366
HAUT27	0.76	0.82	0.4779	0.4154	0.4457	0.4541	0.0152
HEL001	0.63	0.67	0.3075	0.4169	0.3633	0.3683	0.0078
HEL009	0.84	0.79	0.1309	0.1142	0.1228	0.1304	0.0087
HEL51	0.57	0.25	0.3705	0.0537	0.2723	0.3013	0.0399
ILSTS005	0.47	0.20	–0.1852	–0.0160	–0.1344	–0.0986	0.0315
ILSTS006	0.42	0.45	0.8031	0.9730	0.8905	0.8906	0.0011
ILSTS011	0.53	0.61	0.2825	0.5091	0.4057	0.4310	0.0425
ILSTS030	0.64	0.56	–0.0818	0.2008	0.0522	0.0669	0.0155
ILSTS033	0.68	0.62	0.0398	–0.0089	0.0162	0.0188	0.0027
ILSTS034	0.89	0.87	0.2459	0.1195	0.1833	0.1896	0.0077
ILSTS0554	0.68	0.54	0.0388	0.1654	0.0971	0.1081	0.0121
INRA005	0.64	0.68	0.0314	0.2160	0.1271	0.1378	0.0123
INRA035	0.76	0.78	0.3467	0.5915	0.4705	0.4731	0.0050
INRA063	0.70	0.54	0.4166	–0.2990	0.0992	0.1218	0.0250
MM8	0.75	0.68	0.0524	0.0042	0.0291	0.0334	0.0044
MM12	0.81	0.82	0.3722	0.5584	0.4655	0.4674	0.0036
Mean	0.65	0.59	0.2121	0.2248	0.2318	0.2487	0.0219
SD	0.15	0.21					

Gaolao cattle. Means for observed and expected hetero zygosities were 0.47 ± 0.24 and 0.62 ± 0.21, respectively in Kenkatha, and 0.53 ± 0.17 and 0.68 ± 0.14, respectively in Gaolao cattle (table 1).

Test for Hardy–Weinberg equilibrium (HWE) revealed seven microsatellite loci (BM1824, CSRM60, ETH10, ILSTS005, ILSTS033, ILSTS034 and INRA063) in Kenkatha cattle, and three loci (ETH3, ILSTS30 and INRA005) in the Gaolao cattle were in equilibrium where as the remaining microsatellite loci deviated significantly ($P < 0.01$) from HWE. Polymorphic information content (PIC) value for Kenkatha cattle ranged from 0.15 (ETH3) to 0.87 (ILSTS034) with a mean of 0.59 ± 0.21 for all loci, and for Gaolao cattle it ranged from 0.32 (ETH152) to 0.89 (ILSTS034) for all loci with a mean of 0.65 ± 0.15 (table 2). The within–population inbreeding estimate (F_{IS}) ranged between –0.0140 and 0.8031 with an average of 0.2121 in Gaolao cattle, and between –0.0089 and 0.9730 with average of 0.2248 for Kenkatha cattle (table 2).

Fixation indices most currently referred to as *F*–statistics were proposed by Wright to describe the properties of a subdivided population. The mean F_{IS}, F_{IT} and F_{ST} values were 0.2318, 0.2487 and 0.0219, respectively (table 2). Nei's standard genetic distance value between Kenkatha and Gaolao breeds was 0.0852.

Discussion

At least four alleles were detected for each microsatellite locus in both cattle breeds (table 1).This is in agreement with the selective standard of the microsatellite loci given by Barker et al., 1994 and the Secondary Guidelines for Development of National Farm Animal Genetic Resources using reference Microsatellite given by FAO (2004). A minimum of four distinct alleles per locus is proposed for proficient judgment of genetic differences between breeds.

Significant deviation of microsatellite loci studied from HWE (except three loci in Gaolao and seven loci in Kenkatha), and the differences between mean observed and expected heterozygosities within the two cattle breeds suggested a tendency of markers towards heterozygote deficiency and it was reflected in the within–population inbreeding estimate (F_{IS}) for both breeds. Thus, on an average, there is a substantial shortfall of 21.21% and 22.48%, of heterozygotes in Gaolao and Kenkatha populations, respectively. Numerous factors such as inbreeding, genetic hitchhiking, null alleles (nonamplifying alleles) and occurrence of population substructure (Wahlund effect) have been established as reasons for heterozygote deficiency in populations (Nei, 1987). The deviation from HWE, the heterozygote deficiency, and $F_{IS} > 0$ can be attributed to the confinement of Kenkatha and Gaolao breeds to a small geographical area in their respective breeding tract, and a shortage of breeding bulls in the population. Similar observations for a shortage of heterozygotes have been reported in Kherigarh (Pandey et al., 2006) and Tharparkar cattle breeds (Sodhi et al*.,* 2006). The mean observed heterozygosities –Gaolao (0.5350 ± 0.1730) and Kenkatha (0.47 ± 0.24)– found in the present study were lower than the mean heterozygosity reported in Deoni (0.59) (Mukesh et al., 2004) and Kherigarh cattle breeds (0.574) in India (Pandey et al., 2006), and also lower than those shown in seven Italian cattle breeds (0.60−0.68; Del Bo et al., 2001) and five Swiss cattle breeds (0.60−0.69; Schmid et al., 1999). However, in two Indian zebu cattle breeds, whose populations are in rapid decline in India, namely Sahiwal and Hariana (Mukesh et al., 2004), mean heterozygosities and numbers of alleles are lower than in both Gaolao and Kenkatha cattle. Except six loci (BM1824, ETH3, ETH152, HEL51, ILSTS005, ILSTS006) in Kenkatha cattle and five loci (BM1824, CSRM60, ETH152, ILSTS005 and ILSTS006) in Gaolao all other loci possessed a high PIC value (> 0.5), indicating that these markers are highly informative for characterization of both cattle population. Genetic markers showing PIC values higher than 0.5 are normally considered as informative in a population (Botstein et al*.,* 1980). Higher PIC values were also seen in the *taurine* and *indicus* breeds investigated earlier using microsatellite markers (Bradley et al., 1994; Canon et al., 2001; Maudet et al., 2002; Kumar et al., 2003; Metta et al., 2004; Mukesh et al., 2004; Pandey et al., 2006; Sodhi et al., 2006).

Wright's *F*–statistics and other similar indices that describe the partitioning of genetic variance at different hierarchical levels can be estimated for natural populations using a variety of molecular marker data (Nei, 1973). *F*–statistic values F_{ST} and F_{IT} are measures of deviation from Hardy Weinberg proportions and total populations, respectively; where positive values indicate a deficiency in heterozygotes and negative values indicate an excess of heterozygotes. F_{IS} can be interpreted as a measure of inbreeding. Thus, the positive values of F_{IT} (between populations and between the 25 loci in the two breeds) and F_{IS} showed the deficiency of heterozygotes in the populations and that mates were more related in comparison with the average relationship of the population. This observed deficiency of heterozygotes could also be due to non–random sampling. Genetic differentiation between breeds was small. The mean F_{ST} value of 0.0219 showed that the average proportion of genetic variation explained by breed differences was 2.19%, possibly attributable to the geographic distribution of the two breeds. The figure is lower than the 7% of the total genetic variability (mean $F_{ST} = 0.07$) reported by Canon et al. (2001) among local European beef cattle breeds. The Nei's standard genetic distance value between Kenkatha and Gaolao breed also indicated low genetic differention among both breeds. The difference between *Ho* and *He*, $F_{IS} > 0$ and the positive values of *F*–statistics confirmed the deviation from HWE was significant. Thus, from the present study, it was concluded that there is a substantial shortfall, 21.21% and 22.48%, of heterozygotes in Gaolao and Kenkatha cattle populations, respectively; and there is little genetic differentiation (2.19%) between the two breeds.

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