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Molecular characterization of Nimari cattle using fluorescently labeled microsatellite markers

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ABSTRACT

Molecular characterization of Nimari cattle was done for genetic variability study, using fluorescently labeled microsatellite markers as per the recommendation of the FAO and NBAGR, Karnal. Nimari cattle is an important drought-purpose breed of India, which needs urgent conservation due to its declining population in its breeding tract. Number of alleles, allelic frequency, heterozygosity, Hardy-Weinberg equilibrium and polymorphism information content (PIC) revealed higher genetic variability and polymorphism within breed. A total of 206 alleles were observed out of 24 microsatellite markers analyzed with mean of 8.58. Bottleneck analysis revealed no genetic bottleneck in Nimari population.

Key words: Allele frequency, Cattle, Microsatellite, Nimari, PIC

India has diversified and unique cattle genetic resources having 30 well recognized documented breeds (Nivsarkar *et al.* 2000). Microsatellites, highly polymorphic simple tandem repeats are presently the most favoured molecular markers (Litt and Lutty 1989). Microsatellite analysis using fluorescently labeled primers is the pre-eminent method for the genetic analysis of eukaryotic organisms.

Nimari breed of cattle is supposed to be developed by crossing of Gir and Khillari (Tapi valley strain) breeds (Joshi and Phillips 1953). Previously this breed was recognized as a dual-purpose breed, but due to continuous selection for draught power at the cost of milk production, the breed has now developed as an excellent draught breed. Nimari bullocks are excellent for draught purpose so called as 'Biological Engine of Nimar' (Sarkhel, 2001). Nimari breed is found in Nimar region which includes Khargone, Barwani, Khandwa and Harda districts of Madhya Pradesh. Due to the unplanned crossbreeding programme over the years in India, the population of this breed has been declining gradually in the all districts mentioned above. It is also bred in adjacent part of Maharashtra. The present study was planned to study genetic variability of Nimari cattle using 24 fluorescently labeled microsatellite markers (FAO 2004).

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MATERIALS AND METHODS

Study material comprised 70 blood samples of unrelated male and female animals of Nimari cattle, collected randomly from their breeding tract, viz. Barwani and Khargone districts of Madhya Pradesh. Blood sample (10 ml) was drawn from each animal from the jugular vein aseptically in EDTA coated vaccutainer. Blood samples were brought in icebox to the laboratory and stored at –20°C.

DNA was extracted from blood as per John *et al*. (1991). The quality of genomic DNA was assessed by 0.8% agarose gel elecrophoresis using UV transluminator, and purity and concentration of DNA samples were tested by pectrophotometer (ND1000) at optical density (OD) 260nm/ 280nm for subsequent analysis.

Twenty-five microsatellite markers (FAO 2004) were selected to screen population of Malvi cattle. Out of 25, only 24 could be amplified in four multiplex sets (set I carrying 10 microsatellites pairs, set II carrying 6 pairs, Set III carrying 5 microsatellite pairs and set IV carrying 4 microsatellite pairs). The description of primers regarding their chromosomal location, synthesis and labeling are given in Table 1. Sizing was done with ABI 310 genetic analyzer with Liz 500 as international law standard.

Statistical analysis for microsatellite data: All variability parameters were calculated using software microsatellite analyzer version 4.0, downloaded from *http: // i122server.vuwien. ac.at/MSA/info.html/MSA_info.html.* The observed and expected number of alleles, allelic frequency,

Table 1. List of different cattle microsatellite markers used during the study

Marker name	Sequence (5'…….3')	Ta (°C)	Chromosomal Location	5' labeling with Florochrome	Allele size (bp)
BM1818	F: AGCTGGGAATATAACCAAAGG	56	23	ROX	258–272
	R: AGTGCTTTCAAGGTCCATGC				
BM1824	F: GAGCAAGGTGTTTTTCCAATC	58	1	TET	178–192
	R: CATTCTCCAACTGCTTCCTTG				
CSRM60	F: AAGATGTGATCCAAGAGAGAGGCA	55	10	FAM	96–116
	R: AGGACCAGATCGTGAAAGGCATAG				
CSSM663	F: ACACAAATCCTTTCTGCCAGCTGA	60	14	FAM	177–209
	R: AATTTAATGCACTGAGGAGCTTGG				
ETH3	F: GAACCTGCCTCTCCTGCATTGG	60	19	FAM	117–129
	R: ACTCTGCCTGTGGCCAAGTAGG				
ETH10	F: GTTCAGGACTGGCCCTGCTAACA	58	5	FAM	212–224
	R: CTCCCAGCCCACTTTCTCTTCTC				
ETH152	F: TACTCGTAGGGCAGGCTGCCTG	54	5	TET	192–204
	R: GAGACCTCAGGGTTGGTGATCAG				
ETH185	F: TGCATGGACAGAGCAGCCTGGC	56	17	TET	214–246
	R: GCACCCCAACGAAAGCTCCCAG				
HAUT24	F: CTCTCTGCCTTTGTCCCTGT	52	22	HEX	109–129
	R: AATACACTTTATGAGAAAAATA				
HAUT27	F: TTTTATGTTCATTTTTTGACTGG	55	26	HEX	127–155
	R: AACTGCTGAAATCTCCATCCTA				
HEL1	F: CAACAGCTATTTAACAAGGA	54	15	HEX	98–114
	R: AGGCTACAGTCCATGGGATT				
HEL9	F: CCCATTCAGTCTTCAGAGGT	55	8	TET	143–167
	R: CACATCCATGTTCTCACCAC				
HEL51	F: GCAGGATCACTTGTTAGGGA	55	18	FAM	147–171
	R: AGACGTTAGTGTACATTAAC				
ILSTSO11	F: GCTTGCTACATGGAAAGTGC	58	14	FAM	261–271
	R: CTAAAATGCAGAGCCCTACC				
ILSTS030	F: CTGCAGTTCTGCATATGTGG	56	2	TET	149–155
	R: CTTAGACAACAGGGGTTTGG				
ILSTSO33	F: TATTAGAGTGGCTCAGTGCC	56	12	FAM	132–158
	R: ATGCAGACAGTTTTAGAGGG				
ILSTS034	F: AAGGGTCTAAGTCCACTGGC	55	5	HEX	126–150
	R: GACCTGGTTTAGCAGAGAGC				
ILSTS0554	F: GAGGATCTTGATTTTGATGTCC	56	21	FAM	126–150
	R: AGGGCCACTATGGTACTTCC				
INRA005	F: CAATCTGCATGAAGTATAAATAT	55	12	FAM	132–144
	R: CTTCAGGCATACCCTACACC				
INRA035	F: ATCCTTTGCAGCCTCCACATTG	55	16	FAM	102–114
	R: TTGTGCTTTATGACACTATCCG				
INRA063	F: ATTTGCACAAGCTAAATCTAACC	58	18	ROX	171–187
	R: AAACCACAGAAATGCCTTGGAAG				
MM8	F: CCCAAGGACAGAAAAGACT	55	2	FAM	128–150
	R: CTCAAGATAAGACCACACC				
MM12	F: CAAGACAGGTGTTTCAATCT	55	9	TET	105–145
	R: ATCGACTCTGGGGATGATGT				

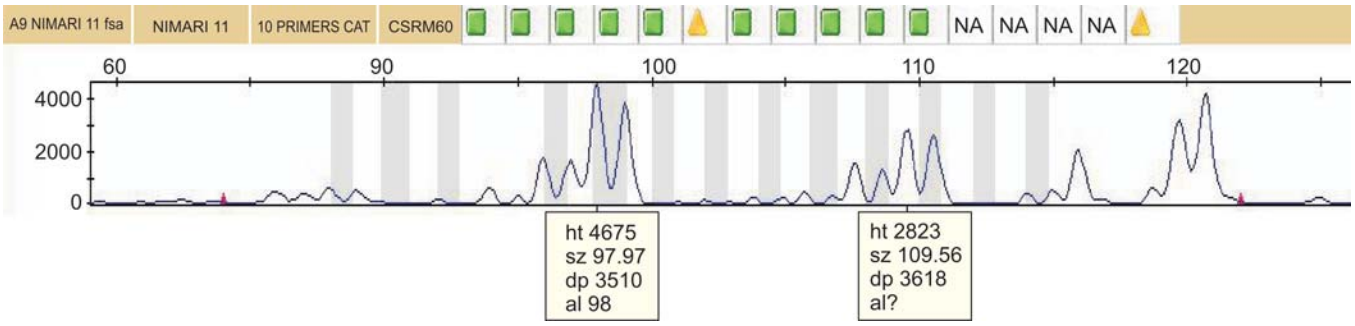

Fig. 1. Electrophoregram showing the alleles found at locus CSRM60.

observed and expected heterozygosity, and Shannon index were estimated using software microsatellite analyzer (version 4.0). Polymorphic information content (PIC) was estimated by using software MS tools (Botstein *et al*. 1980). The program GENEPOP (Raymond and Rousset 1995) was used to compute deviations from the Hardy-Weinberg equilibrium (HWE). The BOTTLENECK program (Piry *et al*. 1999) was applied to determine bottlenecks in the recent past in Nimari population.

RESULTS AND DISCUSSION

Out of 25 fluorescent labeled primers, 24 primers were successfully amplified in 4 multiplexes. In microsatellite analysis all the 24-microsatellite loci were polymorphic in Nimari cattle. A total of 206 alleles were identified across the 24 microsatellites studied. Minimum allele size obtained was of 86 bp and maximum was of 297 bp, which ranging from 3 to 15, with overall mean of 8.58, revealed a reasonable amount of polymorphism. The electrophoregram and allelic frequencies of representative microsatellite loci (CSRM60) are shown in Figs 1 and 2 respectively.

Heterozygosity: Observed heterozygosity values ranged from 0.01 (ILSTS006) to 0.83 (BM1818) while expected heterozygosity ranged from 0.32 (ILSTS011) to 0.87 (HEL9 and INRA063) for all the 24-microsatellite loci used in the present study (Table 2). The overall means for observed and expected heterozygosities were 0.51 and 0.68, respectively, suggesting a tendency of markers towards heterozygote deficiency. Several factors can lead to heterozygote deficiency, including the confinement of the breed to a small geographical area in its breeding tract and shortage of breeding bulls in the population. The mean value for Shannon index was 1.48, which showed the highest genetic diversity within Nimari population.

Polymorphism information content (PIC): PIC value ranged from 0.31 to 0.85 with a mean of 0.64 (Table 2). The highest overall PIC value observed was at microsatellite locus INRA035 (0.85) and lowest at locus ILSTS011 (0.31). Genetic markers showing PIC values higher than 0.5 are normally considered as informative in population (Botstein *et al.* 1980). High PIC value (> 0.5) indicates that these markers are highly informative for characterization of breed. Similar mean PIC

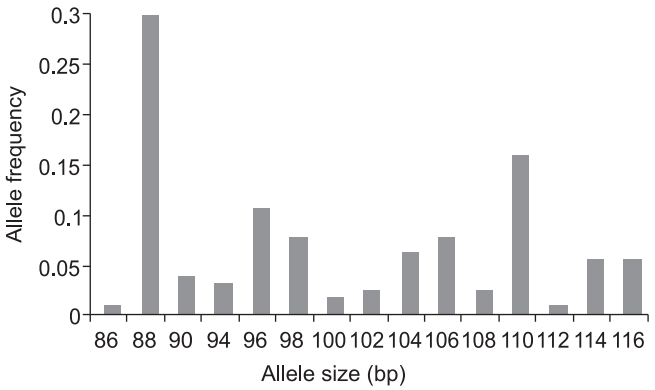

Fig. 2. Allelic frequency distribution at locus CSRM60

value (0.60) was reported by Sodhi *et al.* (2006) for Tharparker breed for 25 microsatellite markers studied and Pandey *et al.* (2006) reported higher PIC value (0.66) in Kherigarh cattle.

Hardy-Weinberg equilibrium (HWE): Microsatellite locus ETH152, HEL1, ILSTS0554, ILSTS033, INRA035, ETH185 and ILSTS006 were found in HWE when tested for Nimari population. Rest of the microsatellite locus showed a significant deviation ($P<0.01$) from HWE. However, microsatellite locus ETH185 and ILSTS006 showed slight deviation from Hardy-Weinberg equilibrium. Thus, population was not in equilibrium as 72% of the loci, deviated significantly ($P<0.01$). All polymorphic microsatellite loci deviated significantly ($P<0.01$) from the HWE. The deviation observed in the population can be attributed to the existence of null alleles, inherently high mutation rate of microsatellies and size homoplasy of loci, besides small population size due to shrinkage of breeding tract and availability of lesser number of bulls. Karthickeyan *et al.* (2006) also reported that 7 loci were in HWE for microsatellite loci screened, and rest of the loci deviated significantly ($P<0.01$) from HWE in Krishna Valley cattle.

Bottleneck analysis: The sign test revealed no bottleneck in the population under infinite alleles model (IAM) and stepwise mutation model (SMM). The results obtained for the standardized difference test in which the T2 values for the two mutation models were lesser, thus null hypothesis of mutation drift equilibrium that was accepted for both the

Table 2. Genetic variability parameters in Nimari breed of cattle

Locus name	Min. allele (size in bp)	Max. allele (size in bp)	Max. obs. allele	Exp. no. allele	Het. obs.	Het. exp.	Shannon ID	PIC
BM 1818	258	282	11	8.59	0.83	0.85	2.06	0.83
BM 1824	176	192	7	5.16	0.49	0.69	1.38	0.64
CSRM 60	86	116	15	9.23	0.60	0.86	2.27	0.84
CSSM663	172	206	14	6.23	0.65	0.77	1.79	0.73
ETH 3	97	121	6	4.75	0.54	0.66	1.26	0.60
ETH 10	205	223	7	5.66	0.68	0.73	1.47	0.68
ETH 152	184	202	8	4.12	0.36	0.58	1.23	0.53
ETH185	217	243	12	5.42	0.15	0.72	1.77	0.69
HAUT27	138	150	6	5.18	0.52	0.70	1.38	0.65
HEL1	115	119	3	3.79	0.71	0.53	0.82	0.42
HEL9	134	166	14	9.41	0.70	0.87	2.15	0.84
HEL51	146	170	10	5.83	0.35	0.74	1.66	0.70
ILSTS005	176	192	7	4.50	0.59	0.63	1.19	0.55
ILSTS006	289	297	5	2.89	0.01	0.37	0.73	0.34
ILSTS011	260	270	6	2.64	0.15	0.32	0.74	0.31
ILSTS030	147	155	5	5.48	0.65	0.72	1.34	0.66
ILSTS033	132	152	7	4.88	0.63	0.67	1.28	0.60
ILSTS034	144	166	12	3.63	0.21	0.50	1.24	0.49
ILSTS054	147	153	4	4.54	0.70	0.63	1.10	0.55
INRA005	132	146	7	6.30	0.72	0.77	1.53	0.72
INRA035	98	118	11	9.52	0.44	0.87	2.13	0.85
INRA063	176	186	6	4.33	0.54	0.61	1.24	0.57
MM8	120	150	12	7.19	0.23	0.81	1.88	0.78
MM12	96	120	11	6.13	0.68	0.76	1.83	0.74
Mean			8.58	5.64	0.51	0.68	1.48	0.64
SD			3.43	1.94	0.22	0.14	0.43	0.15

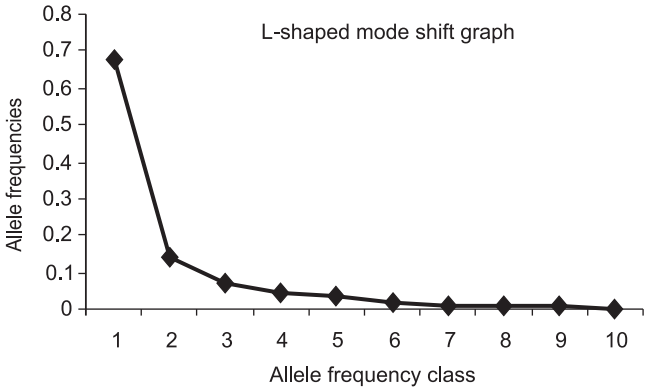

Fig. 3. L-shaped mode shift graph showing lack of recent genetic bottleneck in Nimari population.

mutation models. The Wilcoxon rank tests also revealed similar results under both mutation models, accepting null hypothesis, i.e. no bottlelneck in the Nimari breed (Fig. 3). The mode shift exhibited no distortion of alleleic frequency, and formed normal L-shaped distribution.

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