RESEARCH ARTICLE

Genetic diversity and bottleneck studies in the Marwari horse breed

A. K. GUPTA[1]*, M. CHAUHAN[1], S. N. TANDON[1] and SONIA[2]

[1]*National Research Centre on Equines, Sirsa Road, Hisar 125 001, India*
[2]*Guru Jambeshwar University, Hisar, 125 001, India*

Abstract

Genetic diversity within the Marwari breed of horses was evaluated using 26 different microsatellite pairs with 48 DNA samples from unrelated horses. This molecular characterisation was undertaken to evaluate the problem of genetic bottlenecks also, if any, in this breed. The estimated mean (± s.e.) allelic diversity was 5.9 (± 2.24), with a total of 133 alleles. A high level of genetic variability within this breed was observed in terms of high values of mean (± s.e.) effective number of alleles (3.3 ± 1.27), observed heterozygosity (0.5306 ± 0.22), expected Levene's heterozygosity (0.6612 ± 0.15), expected Nei's heterozygosity (0.6535 ± 0.14), and polymorphism information content (0.6120 ± 0.03). Low values of Wright's fixation index, F_{IS} (0.2433 ± 0.05) indicated low levels of inbreeding. This basic study indicated the existence of substantial genetic diversity in the Marwari horse population. No significant genotypic linkage disequilibrium was detected across the population, suggesting no evidence of linkage between loci. A normal 'L' shaped distribution of mode–shift test, non-significant heterozygote excess on the basis of different models, as revealed from Sign, Standardized differences and Wilcoxon sign rank tests as well as non-significant *M* ratio value suggested that there was no recent bottleneck in the existing Marwari breed population, which is important information for equine breeders. This study also revealed that the Marwari breed can be differentiated from some other exotic breeds of horses on the basis of three microsatellite primers.

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Introduction

India has a complex amalgamation of varying terrains and climatic conditions in which different species of the family *Equidae* are used in diverse roles. On the basis of geographical localisation, six different breeds of horses – Kathiawari, Marwari, Manipuri, Zanskari, Bhutia and Spiti, have been identified in India (National Commission on Agriculture 1976). These breeds of horses are distinct not only because of their adaptation to different agroclimatic conditions prevailing in the country, but also because they have unique performance traits. Marwari horses are known for sturdiness, stiffness, endurance potential, relative disease resistance, and have unique majestic looks much sought after by horse breeders and owners. However, owing to indiscriminate breeding and lack of sound breeding policies, the breed's characteristics are being diluted and presently only a few thousand true Marwari horses are in existence (Singhvi 2001). To avoid further loss of potential unique genes, and to preserve the genetic diversity within the breed, an objective breed classification based on genetic uniqueness is of priority (May 1990; Hall and Bradley 1995). Characterisation at the morphological and genetic levels is the first step towards formulating breeding policies and prioritising the breeds for conservation in an effective and meaningful way. Recently an array of DNA based markers has been developed to carry out studies of genetic variation (Bradley *et al.* 1996; Canon *et al.* 2000). Among these, microsatellites are considered by many to be the most suitable marker system for evaluating breeds for genetic diversity, owing to their abundance in the mammalian genome, high level of polymorphism, codominant inheritance and

*For correspondence. E-mail: akguptanrce@hotmail.com.

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amenability for automation (Takezaki and Nei 1996). Several studies on establishing genetic relationships and differentiation based on microsatellite markers have been reported in livestock breeds, including horses (Arranz *et al.* 2001; Bjornstad and Roed 2001; Fan *et al.* 2002; Ivankovic *et al.* 2002; Kantanen *et al.* 2000). Except for phenotypic characterization, few studies have been carried out in Marwari and other indigenous Indian horse breeds (Yadav *et al.* 2001; Singh *et al.* 2002). The present study involved molecular characterization based on microsatellite markers to detect historical population bottlenecks, if any, in the Marwari horse breed. This is critical as the overall population of these equines has gone down rapidly during the last few decades.

Materials and methods

Blood samples

Blood samples (5–8 ml) from the jugular vein of genetically unrelated healthy individuals (n = 48) of Marwari horse breed belonging to three different equine farms were randomly collected aseptically into vacutainers coated with EDTA (0.5 mM, pH 8.0).

Microsatellite loci

A total of 26 microsatellite markers – HTG2, HTG3, HTG4, HTG5, HTG6, HTG8, HTG20, UM002, UM004, UM005, UM007, UM010, UM011, UM015, UM021, UCDEQ62, UCDEQ502, UCDEQ412, TKY16, TKY19, LEX68, VHL20, VHL123, AHT16, AHT17 and AHT44 (Lindgren *et al.* 1999; Ellegren *et al.* 1992; Meyer *et al.* 1997; Eggleston-Stott *et al.* 1996, 1997; Kakoi *et al.* 1999; Coogle and Bailey 1998; van Haeringen *et al.* 1994, 1998; Swinburne *et al.* 1997, 2000) – reported to be polymorphic in various breeds of horses were analysed to assess the extent of genetic variation in the Marwari breed. The primer pairs were procured from Sigma Aldrich Chemicals Pvt. Ltd, Bangalore, India.

DNA isolation and PCR based profiling

Genomic DNA was isolated from blood samples using standard proteinase K digestion and phenol: chloroform extraction method (Sambrook *et al.* 1989). The purified DNA was quantified, aliquoted in small lots and stored at – 20°C for this study. Polymerase chain reactions (PCR) were carried out in 25 µl reaction containing 100 ng template DNA, 50 ng each primer, 200 µM dNTPs, 10 mM Tris HCl (pH 9.0), 50 mM KCl, 0.01% (w/v) gelatin, and 0.5 U of Taq polymerase (Banglore Genei Pvt. Ltd., Bangalore, India) using Thermal Cycler (Perkin Elmer Corporation, Norwalk, USA). We used 1.5–2.0 mM $MgCl_2$ concentration with different primer pairs (table 1). Thermal conditions for amplification included initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 45 sec, annealing at optimum temperature (table 1) for 45 sec, and extension at 72°C for 1 min each, with a final extension at 72°C for 15 min. PCR products were electrophoresed at 100 volts in a 2% agarose gel, and stained with ethidium bromide (0.5 mg/ml). The amplified products observed under ultra violet light (300 nm) transilluminator were further resolved in 6% urea polyacrylamide denaturing sequencing gel on Sequi-GT system 30 × 38 cm (Bio-Rad Laboratories, Hercules, USA). Alleles were visualized by silver staining, following Bassam *et al.* (1991). The sizes of amplified products were estimated using a 10 bp molecular weight marker (Invitrogen, Life Technologies, New Delhi, India). Genotypes of individual horses at the different polymorphic loci were recorded by direct counting.

Computation and statistical analysis

Allele frequencies for each locus were calculated with $2n$ = 96 for Marwari horses and can be obtained from authors on request. Heterozygosity (Nei 1978) and other genetic diversity variables were calculated using POPGENE computer package (Yeh *et al.* 1999). Polymorphism information content (PIC) values were calculated by using the method described by Botstein *et al* (1980). The probability of random mating in the population was estimated by Chi-square (c^2) and likelihood ratio (G^2) tests to examine Hardy-Weinberg equilibrium (HWE) at each locus. The tests for departure from Hardy-Weinberg proportions and linkage disequilibrium were performed using exact probability tests provided in GENEPOP version 3.1 a (Raymond and Rousset 1999). A Monte Carlo method (Guo and Thompson 1992), with the length of chain set to be 50000 iterations, was used to compute unbiased estimates of the exact probabilities (P values). Ewens–Watterson neutrality test was performed to test the neutrality of microsatellite markers; the statistics for the test were calculated using the algorithm given by Manly (1985), using 1000 simulated samples.

Bottleneck events in the population were tested by three methods. The first method consisted of three excess heterozygosity tests developed by Cornuet and Luikart (1996): (i) Sign test, (ii) Standardized differences test, and (iii) a Wilcoxon sign-rank test. The probability distribution was established using 1000 simulations under three models–Infinite allele model (IAM), stepwise mutation model (SMM) and two-phase model of mutation (TPM).

The second method was the graphical representation of the mode-shift indicator originally proposed by Luikart *et al.* (1998). Loss of rare alleles in bottlenecked populations is detected when one or more of the common allele classes have a higher number of alleles than the rare allele class (Luikart *et al.* 1998). This test was re-scaled so that

frequency distribution of the allele frequency classes in each population would be based on equal 0.05 increments. These two methods were conducted using Bottleneck v1.2.02 (http://www.ensam.inra.fr/URLB).

The third method was the *M* ratio conducted by applying the m_p_val.exe program (Garza and Williamson 2001). The ratio of the number of alleles present at a locus (*k*) to the range of allele sizes in base pairs for the

Table 1. Primer sequences and PCR conditions used for microsatellite loci.

S. No.	Microsat locus	Primer Sequences (Forward & Reverse)	Annealing temperature (°C)	$MgCl_2$ Conc. (mM)	Reference
1.	HTG 2	5′-GATTGGCAACAGATGTTAACTCGG-3′ 5′-CCCCATGAGAACTAACAATGTTAG-3′	55	1.5	Ellegren *et al* (1992)
2.	HTG3	5′TAACCTGGGTGCAAAGCCACCCAT-3′ 5′-TCAGGGCCAATCTTCCTCAC-3′	55	1.5	Ellegren *et al* (1992)
3.	HTG4	5′-CTATCTCAGTCTTGATTGCAGGAC-3′ 5′-CTCCCTCCCTCCCTCTGTTCTC-3′	55	1.5	Ellegren *et al* (1992)
4.	HTG5	5′-TGCTAAGCCTCAG CACATACA-3′ 5′-TGGAAATAAGGTTAGCAGGGATGC-3′	55	1.5	Ellegren *et al* (1992)
5.	HTG6	5′-CCTGCTTGGAGGCTGTGATAAGAT-3′ 5′-GTTCACTGAATGTCAAATTCTGCT-3′	55	1.5	Ellegren *et al* (1992)
6.	HTG8	5′- CAGGCCGTAGATGACTACCAATGA-3′ 5′- TTTTCAGAGTTAATTGGTATCACA-3′	55	1.5	Ellegren *et al* (1992)
7.	HTG 20	5′-CTGGTTTACCTTCCCTACAG-3′ 5′-CCAATGGTTCCTCTGAGAAG-3′	55	1.5	Lindgren *et al* (1999)
8.	UM002	5′-AGTGGCAGCATAAAGATG –3′ 5′-TTTTGGTCCTTGTAGGAG-3′	56	1.5	Meyer *et al* (1997)
9.	UM004	5′-AGGTCAGGTTCACTTTTTC-3′ 5′-AGGTCACTGTGCCTAGTTG-3′	56	1.5	Meyer *et al* (1997)
10.	UM005	5′-CCCTACCTGAAATGAGAATTG-3′ 5′-GGCAAAAGATCAGGCCAT-3′	56	1.5	Meyer *et al* (1997)
11.	UM007	5′-GGGAATAGAGAAAGGTGAAG-3′ 5′-TTAGAGTTCCTGCTCCTCC-3′	56	1.5	Meyer *et al* (1997)
12.	UM010	5′-TACAGCCATTGGAAATCTTAC-3′ 5′-CACCATTACATTTTCCCAG-3′	56	1.5	Meyer *et al* (1997)
13.	UM011	5′-TGAAAGTAGAAAGGGATGTGG-3′ 5′-TCTCAGAGCAGAAGTCCCTG-3′	56	1.5	Meyer *et al* (1997)
14.	UM015	5′-AGTCTGGCTGAGGATACTG-3′ 5′-GGTGAGAAAGGAGATAAATG-3′	56	1.5	Meyer *et al* (1997)
15.	UM021	5′-CGTCCACTTAGGACAATGTAG-3′ 5′-ATGCACAGCAAGATGCAG-3′	56	1.5	Meyer *et al* (1997)
16.	UCDEQ62	5′-AAACTGAGCACCAGACTC-3′ 5′-GATGGATACTCCTGTAGCA3′	55	1.5	Eggleston-Stott *et al* (1996)
17.	UCDEQ502	5′-CCTTGGGCTTTAGCAACT-3′ 5′-CCATTGGAAACTGAGAGG-3′	55	1.7	Eggleston-Stott *et al* (1997)
18.	UCDEQ412	5′-AGAGGAAGGCGACAGGTC-3′ 5′-CATCCGTCCATCCATCAG-3′	55	1.5	Eggleston-Stott *et al* (1997)
19.	TKY16	5′-GGTTATGGTTTGGTATCTGTC-3′ 5′-AAAACAATGGCTTCCTGGTCA-3′	55	1.5	Kakoi *et al* (1999)
20.	TKY19	5′-CTTCTGCTGATTCCTGAATG-3′ 5′-GGATCTCCTTAAATGGAACA-3′	55	1.5	Kakoi *et al* (1999)
21.	LEX68	5′-AAATCCGAGCTAAAATGTA-3′ 5′-TAGGAAGATAGGATCACAAGG-3′	56	1.5	Coogle and Bailey (1998)
22.	VHL20	5′-CAAGTCCTCTTACTTGAAGACTAG-3′ 5′-AACTCAGGGAGAATCTTCCTCAG-3′	60	2.0	van Haeringen *et al* (1994)
23.	VHL123	5′-CCTCCTTCACAGTGAAGTGC-3′ 5′-GAGTATATAGCTCCAGACCTC-3′	57	1.5	van Haeringen *et al* (1998)
24.	AHT16	5′-ATGTTGTGCAAATGGGATGA-3′ 5′-TGCCCATTGATTGATGATTG-3′	60	1.5	Swinburne *et al* (1997)
25.	AHT 17	′- CCCCATAACCACAAGTGAGG-3′ 5′-GAAGTGGGAGAGTCGGTAAGG-3′	60	1.5	Swinburne *et al* (1997)
26.	AHT44	5′-GAAAAGGAGAAAGGATGCCC-3′ 5′-ATGAGAGAGGCCAACCAGG-3′	60	1.5	Swinburne *et al* (2000)

same locus (r) is called the M ratio (M = ¼ k/r; Garza and Williamson 2001). The M ratio is stable if the population size was stable for a long time. When a population experiences a bottleneck event, rare alleles are lost more often by drift than the common alleles during a population size reduction and k is reduced. However, lost alleles do not always occur at the extremes of the allele size distribution so the range in allele sizes (r) will not be reduced at the same rate as k. Consequently the M ratio declines in the event of a bottleneck event. The M ratio was calculated for the whole population and contrasted with that under equilibrium. To test whether an M value is lower than expected, 10000 replicates were simulated. The number of times that the simulated M is higher than the calculated M represents the statistical significance of the M value reduction.

Results and discussion

Genetic characterization of this breed was attempted using known polymorphic microsatellites for studying the genetic variability within the population. Out of 26 microsatellite markers (table 1) found polymorphic in various breeds of horses, three loci (AHT16, AHT44 and UM021) were observed to be monomorphic in Marwari horses. These three markers seem to be in the highly conserved region of the genome and can probably differentiate Marwari breed from the other horse breeds *viz*. Thoroughbred, Arab, Welsh Cob, Icelandic, American Quarter, Standardbred, Hanoverian, Saddlebred, Arab, Polish warm blood, Morgan and Appaloosa horse breeds (Meyer *et al*. 1997; Swinburne *et al*. 1997, 2000). However, the monomorphic nature of these three loci is in agreement with earlier observations on Spiti and Kathiawari breeds of horses (Chauhan 2004; Chauhan *et al* 2004). Marker UM015 could not be scored due to too much nonspecific amplification at this locus. The remaining 22 loci were observed to be polymorphic, and the values of diversity measures at each locus are presented in table 2. A total of 130 alleles were observed at all the loci ranging from 3 (HTG2, HTG4, HTG6) to 12 (UM007), with a mean (± s.d.) of 5.9 ± 2.24 alleles per locus. The mean effective number of alleles in the Marwari horse population was 3.3 ± 1.27, ranging from 1.2 (HTG2) to 7.5 (UM007). The mean observed and expected (Levene's and Nei's) heterozygosities were 0.5306 (± 0.22), 0.6612 (± 0.15, Levene's) and 0.6535(± 0.14, Nei's), respectively. This basic information indicated the existence of high genetic variability within the Marwari equine population. The PIC values suggested that 81.8% markers were highly informative (PIC > 0.5) in terms of their suitability for genetic diversity studies, and the remaining loci were reasonably informative (table 2). The neutrality

Table 2. Observed (n_o) and effective (n_e) number of alleles, allele size range, observed heterozygosity (H_o), expected heterozygosity (H_e), polymorphism information content (PIC), F_{IS} index and average estimates of polymorphic microsatellite loci in Marwari horses.

Microsatellite locus	No. of alleles		Allele size range (bp)	H_o	H_e		PIC	F_{IS} index
	n_o	n_e			Levene's	Nei's		
HTG 2	3	1.2	102–106	0.1875	0.1735	0.1717	0.1600	– 0.0923
HTG3	4	2.9	114–124	0.4000	0.6702	0.6568	0.6056	0.3910
HTG4	3	1.6	128–132	0.1429	0.3933	0.3886	0.3522	0.6324
HTG5	5	2.3	77–97	0.1538	0.5708	0.5634	0.5293	0.7270
HTG6	3	2.7	86–98	0.5714	0.6426	0.6349	0.5629	0.1000
HTG8	6	4.0	176–192	0.5745	0.7575	0.7494	0.7110	0.2335
HTG 20	9	3.9	137–162	0.4091	0.7555	0.7469	0.7149	0.4523
UM002	5	3.4	242–260	0.6591	0.7113	0.7033	0.6560	0.0628
UM004	4	3.4	111–118	0.7234	0.7191	0.7114	0.6611	– 0.0169
UM005	5	3.2	214–222	0.5000	0.6985	0.6912	0.6455	0.2766
UM007	12	7.5	83–132	0.6585	0.8777	0.8670	0.8530	0.2405
UM010	7	3.8	97–121	0.7174	0.7480	0.7398	0.6975	0.0303
UM011	6	3.7	168–189	0.3191	0.7348	0.7270	0.6942	0.5610
UCDEQ62	4	2.2	152–158	0.8542	0.5480	0.5423	0.4427	– 0.5750
UCDEQ502	6	2.0	158–169	0.1915	0.5209	0.5154	0.4830	0.6285
UCDEQ412	8	3.9	190–216	0.5333	0.7501	0.7417	0.7052	0.2810
TKY16	5	2.5	113–129	0.5435	0.6061	0.5995	0.5592	0.0934
TKY19	8	3.0	146–160	0.5833	0.6748	0.6678	0.6375	0.1264
LEX68	8	4.0	148–171	0.8182	0.7631	0.7544	0.7223	– 0.0846
VHL20	6	3.9	89–105	0.6889	0.7498	0.7415	0.7013	0.0709
VHL123	5	3.2	148–163	0.5526	0.6951	0.6859	0.6223	0.1943
AHT 17	8	4.5	107–131	0.8913	0.7855	0.7769	0.7475	– 0.1472
Average	5.9	3.3	–	0.5306	0.6612	0.6535	0.6120	0.2433
SD	2.24	1.27	–	0.22	0.15	0.14	0.03	0.05

of each marker tested by Ewens-Watterson test for neutrality suggested that all the microsatellite loci except HTG6 and UM004 (table 3, observed *F* values lie outside of the upper and lower limits of 95% confidence region of expected *F* values) were neutral and unlinked to any selected trait. The Chi-square (c^2) and likelihood ratio (G^2) tests performed to examine HWE at each locus (table 3) indicated that eleven loci (HTG2, HTG6, UM002, UM004, UM005, UM007, UM010, TKY16, VHL20, VHL123, AHT17; probability ($c^2/G^2 > 0.05$) deviated from HWE. Out of the eleven deviations, only HTG2, UM004, UCDEQ62, LEX68 and AHT17 loci showed higher observed heterozygosity than the expected values (table 2), whereas for the rest of the loci an excess of heterozygotes was observed. Heterozygote deficiency analysis revealed significant deviations from HWE ($P < 0.05$) at some of the loci. It is, however, difficult to envisage the exact basis of this departure, although the presence of low frequency null alleles segregating at these loci may be a possible reason. An exact test for genotypic linkage disequilibrium yielded no significant *P* values across the population, and therefore independent assortment was assumed.

The mean value of within-population inbreeding estimates ($F_{IS} = 0.2433 \pm 0.05$) indicated the low level of inbreeding in the population (table 2). High levels of heterozygosity, PIC and low level of heterozygosity deficit in the Marwari horse population reflect high genetic variability that can be exploited by horse breeders for planning breeding strategies and prioritizing the breed for its conservation.

Since the population of Marwari horses true to their breed has gone down drastically and only a few thousands are available (Singhvi 2001), it is possible that demographic bottlenecks might have occurred. Because bottlenecks influence the distribution of genetic variation within and among populations, the genetic effects of reductions in population size require evaluation. In the present study, evidence for a bottleneck was not detected with any of the three methods. To characterize this, Sign, Standardized differences and Wilcoxon sign rank tests were utilized. The values of average heterozygosity (H_e) and their probabilities ($H > H_e$) in the Sign test, under three models of microsatellite evolution – IAM, SMM and TPM – were calculated and used to measure the expected number of loci with heterozygosity excess which was 12.63 for IAM under null hypothesis (table 4). The probability value in this case was 0.00071 and thus rejects the null hypothesis indicating bottleneck under this model. However the expected number of loci with heterozygosity excess were 12.96 and 13.09 in TPM and SMM with probabilities 0.13497 and 0.13047 respectively, meaning that the null hypothesis was accepted when using the Sign test. These results indicate that, due

Table 3. Chi-square and G square probabilities (95% confidence level), observed *F*, and upper (U95) and lower (L95) 95% confidence limits of expected *F* values across 22 polymorphic loci in Marwari horses.

	Hardy Weinberg Equillibrium test			Ewens-Watterson Neutrality test		
Microsat Locus	Degrees of freedom	Probability Chi-square	Probability G-square	Obs. *F*	L95	U95
HTG2	3*	0.929242	0.842470	0.8283	0.3622	0.9590
HTG3	10*	0.000941	0.000984	0.3432	0.2856	0.8488
HTG4	6	0.000000	0.000020	0.6114	0.3710	0.9532
HTG5	10	0.000000	0.000000	0.4366	0.2650	0.8307
HTG6	3*	0.199843	0.161955	0.3651**	0.3625	0.9532
HTG8	15	0.000005	0.005418	0.2506	0.2343	0.7660
HTG20	36	0.000228	0.012366	0.2531	0.1617	0.5444
UM002	10*	0.035159	0.017173	0.2967	0.2663	0.8301
UM004	6*	0.078422	0.039256	0.2886**	0.3010	0.9174
UM005	10*	0.000368	0.000083	0.3088	0.2602	0.8427
UM007	66*	0.428452	0.624024	0.1330	0.1255	0.4191
UM010	21*	0.830766	0.780070	0.2602	0.2030	0.6730
UM011	15	0.000000	0.000000	0.2730	0.2254	0.7673
UCDEQ62	6	0.000482	0.000018	0.4577	0.3101	0.9191
UCDEQ502	15	0.000000	0.000017	0.4846	0.2349	0.7673
UCDEQ412	28	0.000000	0.011087	0.2583	0.1805	0.6131
TKY16	10	0.143499	0.389340	0.4005	0.2644	0.8360
TKY19	28	0.034170	0.380092	0.3322	0.1838	0.5931
LEX68	28	0.000000	0.520436	0.2456	0.1844	0.6229
VHL20	15*	0.198776	0.105168	0.2585	0.2353	0.7558
VHL123	10*	0.113992	0.083735	0.3141	0.2497	0.8269
AHT17	28*	0.819029	0.614369	0.2231	0.1824	0.5952

*Deviations from HWE; **Deviations from neutrality.

Table 4. Test for null hypothesis under three microsatellite evolution models.

Test/Model	IAM	TPM	SMM
Sign test: number of loci with heterozygosity excess (probability)			
	Expected = 12.63 (0.00071)*	12.96 (0.13497)	13.09 (0.13047)
	Observed = 20.00	16.00	10.00
Standard differences test: T_i values (probability)			
	3.689(0.00011)*	1.614(0.05326)	– 1.997 (0.02289)*
Wilcoxon rank test (probability of heterozygosity excess)			
	0.00003*	0.03289*	0.17619

*Rejection of null hypothesis/bottleneck.

to mutation-drift equilibrium, the Marwari population has not undergone a recent genetic bottleneck.

The standardized difference test provided the T_2 (probability) statistics equal to 3.689 (0.00011), 1.614 (0.05326) and – 1.997 (0.02289) for the IAM, TPM and SMM models, respectively. The probability values were less than 0.05 for IAM and SMM, thus hypothesis of mutation-drift equilibrium was accepted under TPM only. Using the Wilcoxon rank test (a non-parametric test) the probability values were 0.00003 (IAM), 0.03289 (TPM) and 0.17619 (SMM) under these three models, indicating that the null hypothesis is accepted under SMM only and the population under study has not undergone a recent bottleneck. Taking results from all the three tests together, it is clear that serious demographic bottlenecks have most probably not occurred in this breed.

The Mode-shift indicator test was also utilized as a second method to detect potential bottlenecks, as the non-bottleneck populations that are near mutation-drift equilibrium are expected to have a large proportion of alleles with low frequency. This test discriminates many bottle necked populations from stable populations (Luikart 1997; Luikart and Cornuet 1997). A graphical representation utilizing allelic class and proportion of alleles showed a normal 'L' shaped distribution (figure 1) This distribution clearly reinforces the result that the studied population has not experienced a recent bottleneck. The *M* ratio for Marwari population was 0.7234 and this value was not statistically significant at the 0.05 level, further indicating the absence of bottleneck events in the recent past history of this breed.

The present work contributes to the knowledge of population structure and assessment of existing genetic diversity in the Marwari horse population. Further genetic analysis of other Indian horse breeds and their comparisons need to be carried out to determine the phylogenic evolutionary relationships and genetic distances among the indigenous equine breeds. The strong inference that the Marwari breed has not undergone major bottlenecks is also important for equine breeders and conservationists, as it suggests that any unique alleles present in this breed may not have been lost.

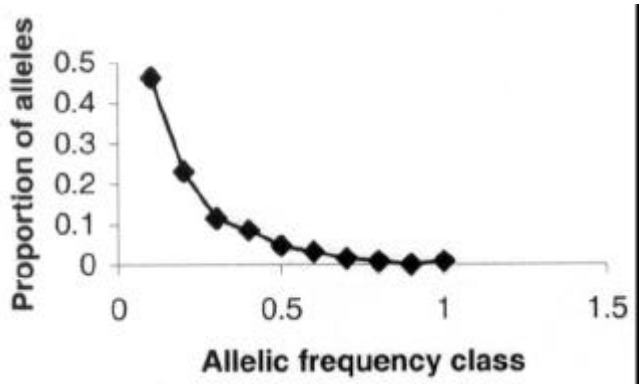

Figure 1. Graphic representation of proportion of alleles and their distribution in Marwari horses.

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