Genetic variability and conservation value flaunt in donkey population of hot and semiarid region of India

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Abstract

The characteristic of donkey population of Marathwada region is grey to brown coat colour and typical features include white belly, eye-ring, area around the muzzle and inner part of fore and hind limb. In present study, a panel of ten microsatellite markers *viz*., AHT5, HTG6, HTG7, HTG10, HMS2, AHT4, NVHEQ54, COR18, COR7 and COR71 were successfully amplified and used for defining genetic variability in the population. The mean allelic richness was 5.44 ± 1.72, which indicates a considerable amount of variability in the population. The mean *Ho* and *He* for the population was 0.694 and 0.802 respectively**.** Heterozygosity value (H) (0.802) also indicated copious genetic variability and suggest that this genetic resource has a high conservation value. All the ten markers supplemented ample degree of information that is indicative from the Shannon index (I) value of 1.915 with evenness 0.831. The population was found to deviate significantly from HW proportions at all loci ($p < 0.0001$) except for HMS2. Polymorphic information content (PIC) value for the markers ranged from 0.663 (COR7) to 0.87 (HTG10) suggesting the suitability of these markers for genetic variability studies in the donkey population of Marathwada region. There was absence of any recent genetic bottlenecks in the studied population and a PE value of 1 indicated suitability of these 10 loci for parent exclusion studies in the population. The study suggests that the population of donkey in Marathwada region has a potential to be recognized as a breed.

Key words: Microsatellite; genetic characterization; donkey; diversity; Marathwada

Introduction

In Marathwada, the donkeys are traditionally reared by washer-man community and pot makers. The major utility of these animals is for carrying sand from the river bed and transportation of silted clay soil for preparation of bricks, shipping of bricks, agricultural products and by-products, including grains and straw, roughages *etc*. Donkeys find their place in the cultural heritage of Marathwada too, though no attempt has been made to classify these animals as breeds. After initiation of "*Mission towards Zero Non-Descript AnGR of India",* by NBAGR in Aug 2021(Annual Report, 2020, https://nbagr.res.in), we make an attempt to study genetic variability of these animals which is a step towards identification of Donkey population of Marathwada as a breed.

The typical features of these donkeys include grey to brown coat colour (Fig 1). Some are white in colour. A dorsal line on the back starting from the head up to tail is prominent in grey or brown coloured animals. Other features include white ring around eye and muzzle. The area on the belly and between fore legs and hind legs is white in colour. Ears shows a marked dark outline. The area around the lower jaw is also white in colour (Fig 2).

Molecular markers, primarily microsatellite markers are proven tools used to determine genetic diversity and relationship within and between animals. Many workers efficiently used microsatellite markers for revealing genetic variation among various donkey breed (Zhang *et al*. 2016; Yun and Cho. 2017; Sharma *et al*. 2017; Yatkin *et al*. 2020; Kefena *et al.* 2021; Bhel *et al*. 2017a, Bhel *et al*. 2017b, Bhel *et al*. 2019, Bhel *et al*. 2021). With increased mechanization and reduction trend in donkey population all over India, it was felt that if the present population is registered and welfare schemes are implemented, the donkey population of the area will augment for sure. With this motive, the study was proposed to analyze simple sequence repeats (SSR) or microsatellite data and genetic variability of donkey population in the region.

Material and Methods

Blood samples were collected from 35 unrelated donkeys (27 Males and 8 Females) predominantly gray to brown in colour by convenient sampling method from five villages of Latur and Nanded district of Marathwada region of Maharashtra. Genomic DNA was isolated using a blood DNA isolation kit (QIAamp DNA Blood Mini Kit) as per standard protocol. DNA samples were stored at -20ºC and dilutions were taken as per requirement. The genomic DNA was amplified by PCR using a set of ten heterologous microsatellite marker primers labeled with HEX (green), FAM (blue), Cy3 (Orange) and Cy5 (far-red) fluorescent dyes (FAO, 2011) used for microsatellite analysis in horse.

Each 20µl PCR reaction, consisting of DNA (about 100 ng), primers (0.2µM each forward and reverse), dNTPs (200 µM each), 10X buffer (50 mM KCl, 10mM tris-HCl, 0.1% gelatine), $MgCl_2$ (1.5 mM) and Taq DNA polymerase (1 unit) was subjected to touchdown PCR protocol including an initial denaturation at 95ºC for 5min followed by 32 cycles of denaturation, annealing and extension (95 ºC for 45 sec, annealing temperature for 30 sec, 72 ºC for 45 sec). The touchdown protocol included step wise reduction in annealing temperature starting from 4 cycles of 60 ºC, four cycles of 56 ºC and 24 cycles at 52 ºC. Final extension was carried out at 72 ºC for 5mins. Each 1µl of PCR products mixed with 0.25 µl of size standard fluorescent dye GS Liz 500 (Applied Biosystems) and Hi-Di formamide were run on ABI Prism 310® capillary based genetic analyzer for differentiation of allele.

Genotypic table were extracted with the help of Gene mapper and peak scanner software. All variability parameters and genetic distances were calculated using Microsoft Excel (MS-Excel). The PIC content was derived using online calculator on (https://gene-calc.pl/pic). The bottleneck analysis was performed (Allendorf. 1986). The observed heterozygosity (Ho) and expected heterozygosity (He) at each locus were calculated by using allele frequencies p and q by calculating the expected frequencies of the homozygous dominant, heterozygous and homozygous recessive individuals. The expected number of alleles and heterozygosity value were calculated using formulas.

Expected number of alleles (Ne) = $N_e = \frac{1}{1-H_{exp}}$

Heterozygosity value (H) = $H = 1 - \sum_{i=1}^{l} \text{Pi}$

For HW equilibrium observed and expected values of the genotypes were compared using the Chi-square goodness of fit analysis using following formula:

$$\chi 2 = \sum_{i=1}^{n} \frac{(Oi-Ei)}{Ei}$$

Fixation index *i.e.,* Fis values were calculated using the formula $Fis = \sum_{i=1}^{n} \frac{(E_i-O_i)}{E_i}$

Where, Oi – observed value, Ei – expected value and n – a set of all alleles

Fig 1. Coat colour of Marathwada donkey varying from grey, light brown to dark brown

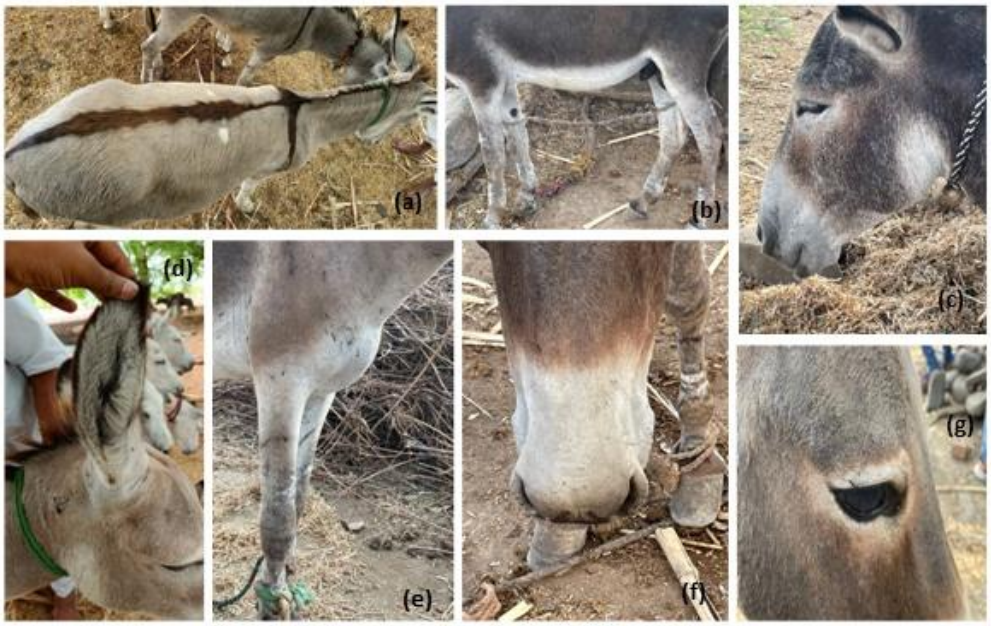

Fig 2. Typical features of Marathwada donkey (a) dorsal line from head to tail (b) white colour of belly (c) white colour on lower jaw (d) black marked outline on ear (e) white marking inside foreleg (f) white marking around muzzle (g) white marking around eye

Results

All ten microsatellite loci *viz*., AHT5, HTG6, HTG7, HTG10, HMS2, AHT4, NVHEQ54, COR18, COR7, COR71amplified in 35 samples. The PCR product visualized different product size for each locus (table 1). The number of alleles observed for different microsatellite loci were 13, 7, 9, 11, 6, 12, 12, 10, 8 and 16 for locus AHT5, HTG6, HTG7, HTG10, HMS2, AHT4, NVHEQ54, COR18, COR7 and COR71 respectively. Highest number of alleles with widest distribution of size was obtained for locus COR71 (16) while less allele were observed for locus HMS2 (6) (table 1). Genetic variability study revealed that *No* or number of observed alleles ranged from 6 in HMS2 to 16 in COR71. The mean number of allele per locus was 10.4 and total number of alleles in the population was 104. The effective number of alleles ranged from 3.07 for COR7 to 7.82 for HTG10. The mean allelic richness in the present study was 5.44 ± 1.72. (table 1)

The Heterozygosity value (H) calculated for the donkey population studied was 0.802. Highest H value was observed 0.872 for locus HTG10 and minimum was observed for COR7 *i.e.,* 0.674. The observed heterozygosity (*Ho*) ranged from 0.348 (AHT4 and NVHEQ54) to 0.932 (AHT5). The expected heterozygosity (*He*) was a minimum of 0.33 for COR71 locus and maximum value was 0.872 for HTG10 locus. A higher value of *Shannon diversity index* (*I*) was contributed by COR71 (2.23) and the lowest was contributed by HMS2 (1.48) (table 1). The population was found to deviate significantly from HW proportions at all loci ($p < 0.0001$) except for HMS2 locus.

Polymorphic information content (PIC) value of the population was **0.797.** The locus HTG10 was found to be more polymorphic while (PIC 0.87) while COR7 (PIC 0.663) was least polymorphic in the population studied. Probability of parentage (P) value, for the locus ranged from 0.673 for COR7 locus to 0.899 for HTG10 locus (table 1). The cumulative probability of parentage exclusion **(**PE) estimated for the locus in the population (PE = 1 – (1–P1) (1–P2) (1–P3) ... (1–PK)) was 1. The allelic diversity (A) for each locus was around 1.

Table 1 Characteristics for 10 microsatellite loci in donkey population of Marathwada

	Allele size range	**N_o**	**N_e**	**H**	**H_o**	**H_e**	**I**	**Fis**	**PIC**	**PE**	**En'**	**A**
AHT5	80- 201	13	6.51	0.846	0.932	0.846	2.14	-0.4	0.845	0.877	12.99	0.999
HTG6	55-85	7	4.77	0.79	0.914	0.79	1.68	-1.8	0.785	0.775	6.999	0.999
HTG7	56- 143	9	4.37	0.771	0.814	0.771	1.7	-1.7	0.759	0.754	8.998	0.999
HTG10	80-103	11	7.82	0.872	0.927	0.872	2.18	-0.1	0.87	0.899	10.999	0.999
HMS2	224-239	6	3.66	0.727	0.625	0.727	1.48	0.3	0.713	0.69	5.999	1
AHT4	116-161	12	6.23	0.84	0.348	0.84	2.15	0.1	0.839	0.88	11.999	0.999
NVHEQ54	182-204	12	6.23	0.84	0.348	0.84	2.15	0.1	0.839	0.88	11.999	0.999
COR18	252-270	10	5.71	0.823	0.84	0.825	1.94	-1.1	0.82	0.835	9.999	0.999
COR7	165-178	8	3.07	0.674	0.565	0.674	1.5	-0.4	0.663	0.673	7.999	0.999
COR71	75-210	16	6.04	0.834	0.632	0.33	2.23	-0.5	0.833	0.876	15.998	0.999
Mean/ Cumulative		10.4	5.44	0.802	0.694	0.802	1.915	-0.55	0.797	1	10.398	1

PCR product size range (bp), observed (N_o) and effective (N_e) number of alleles, heterozygosity (H), observed (H_o) and expected (H_e) heterozygosity, Shannon diversity index (I), Fixation index (Fis)**,** polymorphism information content (PIC), Probability of parentage exclusion (PE), Bottleneck effect (En') and Allelic diversity after a bottleneck (A)

Discussion

The equines are still being used in India as draft animal providing source of earning for poor rural people (Bhat et al., 2022). A wider range of allele size and more number of alleles was reported for locus AHT5 in Marathwada population when compared to Rajasthan, Andhra Pradesh, and Ladakh donkey populations (Sharma *et al*. 2017; Bhel *et, al*. 2017b, Bhel *et al*. 2019). HTG6 and HTG7 loci were found to be small sized *i.e.,* 55-85 bp and 56-143 bp respectively in our study when compared to the allele size in other Indian donkey population wherein the size ranged from 80-100 bp and 120- 150 bp on an average. Locus NVHEQ54 was found to contribute 12 alleles, which is more as compared to other donkey populations of India- (Sharma *et al*. 2017; Bhel *et al.* 2017a, Bhel *et al*. 2017b, Bhel *et al*. 2019, Bhel *et al*. 2021). Widest range of allele size along with maximum number of alleles was seen for the locus COR71 in the studied population when compared to the donkey population of Himachal Pradesh, Andhra Pradesh (Bhel *et, al*., 2017b), Rajasthan (Sharma *et al*. 2017), Ladakh (Bhel *et al.* 2019) and Uttar Pradesh (Bhel *et, al*. 2021) region of India. Study proved that, locus HTG6, HTG7, NVHEQ54 and COR71 are instrumental to differentiation of Marathwadi donkey population from rest of the Indian donkey populations.

The mean allelic richness in the present study was 5.44 ± 1.72 which indicates a considerable amount of variability in the population. Heterozygosity values obtained in the study are in accordance with the values obtained by other researchers in Indian Donkey population. The observed heterozygosity was lower at locus HMS2, AHT4, NVHEQ54 and COR7 indicating some level of inbreeding attributed to small flock size and progressive reduction in

the number of animals. The Shannon diversity index (I) value indicated that the microsatellite locus considered for the study contributed characterizing the genetic variability of the population under study efficiently.

Deviation of the population from Hardy-Weinberg's (HW) equilibrium is evident in the study as also indicated in the study conducted by Bhel *et al*. 2021, wherein five out of ten loci deviated from HW proportions. The stakeholders in present study merely practiced selective breeding of the donkeys, though as evident from the values for *Ho* and *He*, there could have been inbreeding due to small flock size which led to deviation of the population from HW equilibrium at considerable number of locus. Fis value signified that seventy percent of the loci illustrate heterozygosity excess (AHT5, HTG6, HTG7, HTG10, COR18, COR7 and COR71) with significance at all these loci. Presence of heterozygosity excess demonstrated an absence of Wahlund effect at these loci indicating absence of sub-structuring of population. Similar type of results was demonstrated in a study conducted in Rajasthan Donkey population by Sharma *et al*. (2017).

PIC value suggested the suitability of these markers for genetic variability studies in the donkey population of Marathwada. This panel of ten microsatellite loci have proved to be resourceful in diversity studies conducted on other Indian Donkey populations too (Sharma *et al.* 2017; Bhel *et al*. Bhel *et al*. 2017a, Bhel *et al*. 2017b, Bhel *et al*. 2019, Bhel *et al*. 2021). PE indicates the suitability of these ten loci for parent exclusion study in the population as also signified by other researchers in India (Sharma *et al*. 2017; Bhel *et al*. 2017a, Bhel *et al*. 2017b, Bhel *et al*. 2019, Bhel *et al*. 2021). Bottle neck analysis demonstrates absence of any recent genetic bottlenecks in the studied population as also seen in other Indian donkey population (Sharma *et al*. 2017; Bhel *et al*. Bhel *et al*. 2017a, Bhel *et al*. 2017b, Bhel *et al*. 2019, Bhel *et al*. 2021). This proves that irrespective of progressive decline in donkey population of India, there has not been a departure of population from mutation drift equilibrium and that there is copious genetic variability in the population.

Conclusions

The study indicated maximum allelic richness in the population under study as compared to all the other donkey population of India. Genetic diversity exhibited in the population under study is a positive sign for long-term survival of the population as this diversity serves to be the raw material for adaptation and evolution in changing environmental conditions. Our study lays a foundation for recognition of the donkey population of Marathwada region as a breed so that the conservation and welfare strategies for these genetic resources could be planned.

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