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Genetic characterization of Mewari chicken based on microsatellite markers

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Abstract
The present study was undertaken for evaluation of genetic diversity of indigenous chicken Mewari of southern and central Rajasthan by using 24 microsatellite markers in eight multiplexed set. A total of 245 alleles were found in present study where the observed number of alleles per locus varied from 3 (ADL 210) to 21 (ADL 136). The mean observed numbers of alleles were found to be 10.21. The overall means for observed and expected heterozygosity were 0.560 and 0.810 respectively, with the ranges of 0.182 (ADL 39) to 0.927 (MCW 59) and 0.601 (ADL 210) to 0.926 (ADL 136), respectively. The Fixation index (FIS) value for all 24 microsatellite loci ranged from -0.088 (ADL 210) to 0.757 (ADL 39) with mean of 0.280. Polymorphic Information Content (PIC) value was ranging from 0.503 (HUJ 2) to 0.949 (ADL 136) with a mean of 0.813. The test for genetic equilibrium indicated that out of total 24 microsatellite loci, 22 microsatellite loci were found deviated significantly from Hardy-Weinberg Equilibrium. The high number of observed alleles and high heterozygosity indicated presence of high genetic variability in Mewari chicken which is indigenous native of southern and central Rajasthan.

Keywords: Microsatellite, genetic characterization, Mewari chicken, heterozygosity

Introduction
The modern chicken's ancestor can be linked back either to wild red jungle fowl (monophyletic origin) or to wild jungle fowl species of the genus *Gallus* of the family Phasianidae, namely *Gallus gallus* (Red jungle fowl), *Gallus sonneratii* (Grey jungle fowl) found in Southwest India, *Gallus lafayetii* (Ceylon jungle fowl) found in Sri Lanka, and *Gallus varius* (Green jungle fowl) endemic to Java and neighbouring islands [1]. Among the chicken breeds, indigenous or local breeds mostly contribute to the world's poultry genetic diversity in varied geomorphological region [2].

The rearing of indigenous chickens plays an important role in economic and nutritional aspects of rural as well as tribal families who keep poultry in India, mainly due to their adaptability to different agroclimatic conditions [3]. Backyard poultry farming is a part of the typical rural/tribal household, touching on social, cultural, and economic aspects in India. Indigenous chicken has an inherent scavenging and nesting habit. Many years of natural selection under scavenging conditions made them tough and resistant to various diseases, caused by bacteria, protozoa, other internal and external parasites. They have better survival than the commercial hybrid strains under village production conditions [5].

The village chicken is invariably a coloured bird. The colour can be brown, yellow, black or a mixture of these. Multiple colours might serve as camouflage against aerial predators. The village chickens are very alert and have long shanks to run away from predators. He can incubate their eggs and brood their chicks. This enables them to reproduce without any assistance [6].

Genetic diversity forms the basis for breed improvement. The livestock populations are resilience to changing environments and demands. The construction of strategies for the long-term management of animal genetic resources (AnGR) requires an understanding of their origin and history [7]. In developing nations, the threat of local chicken populations losing genetic diversity and/or specific features through inbreeding or crossbreeding has become a significant issue [8]. Local and/or indigenous chicken breeds, which have evolved via centuries of adaptation, domestication, and breeding, are a valuable source of genes for future breeding and research. The group of birds serves as a great library of genotype variability.

Microsatellites or simple sequence repeats (SSRs), or short tandem repeats (STRs), discovered in 1984, are tandemly repeated motifs of 1-6 nucleotides found in all eukaryotic genomes.

In addition to being highly variable and polymorphic, microsatellites are also easy to genotype and densely distributed throughout eukaryotic genomes, making them the preferred genetic marker for high resolution genetic mapping. With the advancement in sequencing and genotyping technologies, it has now become much easier to genotype microsatellite loci in large number of samples in a short span of time. Microsatellites are used as molecular markers in poultry research, specifically in some genetic resources of economically important species such as chickens, quails, ducks, geese, turkeys, and other birds [10].

The Indian chicken population consist of different phenotypes of almost centuries of natural selection and reared by small holder farmers across distinct agro-ecological regions. Investigations on the use of microsatellite markers to evaluate the genetic variability of these ecotypes are limited though a large number of microsatellite primers are available for diversity studies. Despite the importance of native chickens in tribal/rural areas, there is a paucity of data regarding their genetic makeup, especially genetic variability, genetic relations, performance, resilience, and disease resistance [11]. Hence, the present study was conducted to evaluate genetic diversity among Mewari chicken population.

Materials and Methods

A total of sixty blood samples were collected randomly from non-related chickens from five tehsils Badgaon, Jhadol, Mavali, Nathdwara, Rajsamand of three districts viz., Udaipur, Rajsamand, Bhilwara from central and Southern part of Rajasthan.

The 24 microsatellite primers were selected based on their fragment size and fluorescent dye label (Table 1). PCR amplification of 24 microsatellite loci was done into seven multiplexed set. The genotyping was performed using ABI PRISM 3500 Genetic Analyser, automated DNA Sequencer and Gene Mapper software version 4.1 (Applied Biosystem, USA). The statistical analysis was performed using Gene Alex version 6.503.

Table 1: Primers used for PCR amplification and Multiplex PCR set for amplification of microsatellite loci of Mewari

Sr. No	Markers Name		Sequence (5' 3')	Annealing Temperature (°C)	5' Labelling with	Chromosomal Location Linkage Group	Multiplex PCR Set
1	HUJ 2	F	CATCTCACAGAGCAGCAGTG	57	FAM	17	Set-I
	HUJ 2	R	GAATCCTGGATGTCAAAGCC		FAM	5	
2	ADL 23	F	CTTCTATCCTGGGCTTCTGA	57	HEX	9	
	ADL 23	R	CCTGGCTGTGTATGTGTTGC		TET	1	
3	ADL 136	F	TGTCAAGCCCATCGTATCAC	57	TET	8	
	ADL 136	R	CCACCTCCTTCTCCTGTTCA		HEX	10	
4	LEI 146	F	TCAAGCCACCAAAGTGCTTGG	57	FAM	2	
	LEI 146	R	GATCACTCTGCTCATAGCAGT		FAM	C3E6	
5	HUJ 12	F	TAAAATTTATCTTTGAAAATGCCT	57	FAM	C4E28	
	HUJ 12	R	GAGAAACATGTATTTCCAATTATTC		FAM	3	
6	ADL 158	F	TGGCATGGTTGAGGAATACA	57	TET	C3E6	Set-II
	ADL 158	R	TAGGTGCTGCACTGGAAATC		TET	C1E2	
7	ADL 176	F	TTGTGGATTCTGGTGGTAGC	57	FAM	1	
	ADL 176	R	TTCTCCCGTAACACTCGT		TET	15	
8	ADL 267	F	AAACCTCGATCAGGAAGCAT	57	FAM	12	
	ADL 267	R	GTTATTCAAAGCCCCACCAC		FAM	11	
9	MCW 1	F	ACTGTCACAGTGGGGTCATGGACA	54	HEX	1	
	MCW 1	R	ACACGTCCTGTGTCACATGCCTGT		FAM	10	
10	MCW 16	F	ATGGCGCAGAAGGCAAAGCGATAT	54	HEX	E42	Set-III
	MCW 16	R	TGGCTTCTGAAGCAGTTGCTATGG		FAM	1	
11	MCW 51	F	GGAACAAGCTCTTTCTTCTTCCCG	54	HEX	20	
	MCW 51	R	TCATGGAGGTGCTGGTACAAAGAC		FAM	C3	
12	MCW 59	F	AAGTGCCTTTGCTATCCTGATTGG	54	TET	C10,E36	
	MCW 59	R	AACTCCTATTGTGCAGCAGCTTAT		FAM	2	
13	MCW 7	F	AGCAAAGAAGTGTTC TCTGTTCA	59	FAM	17	Set-IV
	MCW 7	R	ACCCTGCAAACTGGAAGGGTCTCA		FAM	5	
14	ADL 39	F	GCTACAACGCTTCAAACCTG	57	HEX	9	Set-V
	ADL 39	R	ACAAACAAACCAAAAAACCT		TET	1	
15	ADL 44	F	AAGTGGTTTATTGAAGTAGA	57	TET	8	
	ADL 44	R	CTGTGGTGTTGCGTTAGTTG		HEX	10	
16	ADL 210	F	ACAGGAGGATAGTCACACAT	57	FAM	2	
	ADL 210	R	GCCAAAAAGATGAATGAGTA		FAM	C3E6	
17	MCW 11	F	TAAAATTTATCTTTGAAAATGCCT	48	FAM	C4E28	Set-VI
	MCW 11	R	GAGAAACATGTATTTCAATTATTC		FAM	3	
18	ADL 102	F	TTCCACCTTTCTTTTTTATT	48	TET	C3E6	
	ADL 102	R	GCTCCACTCCCTTCTAACCC		TET	C1E2	
19	ADL 172	F	CCCTACAACAAAGAGCAGTG	48	FAM	1	
	ADL 172	R	CTATGGAATAAAATGGAAAT		TET	15	
20	MCW 43	F	TGACTACTTTGATACGCATGGAGA	57	FAM	12	Set-VII
	MCW 43	R	CACCAAGTAGACGAAAACACATTT		FAM	11	
21	ADL 34	F	AACCTAAAAACTCCTGCTGC	57	HEX	1	
	ADL 34	R	GGGAACCTGTGGGCTGAAAG		FAM	10	

22	LEI 65	F	TGAAACATGTATGGAGTCTCAGCA	57	HEX	E42	
	LEI 65	R	GACAGCTAAATGCCAGTTCATGG		FAM	1	
23	MCW135	F	ATATGCTGCAGAGGGCAGTAG	58	HEX	20	Set-VIII
	MCW135	R	CATGTTCTGCATTATTGCTCC		FAM	C3	
24	MCW312	F	TTTGTTCGGGATTAAGCTTGG	58	TET	C10,E36	
	MCW312	R	CCTAAATCAGGATGTTTGGAC		FAM	2	

Results and Discussion

Observed and effective number of alleles

A total of 245 alleles were identified across the 24 microsatellites in Mewari chicken. The number of observed alleles per locus among the polymorphic markers ranged from 3 (ADL 210) to 21 (ADL 136) in Mewari chicken indicating considerable variation in the distribution of allele frequencies between loci observed in population. The effective number of alleles (Ne) is the best measures of genetic variation and it is usually lower than the observed number in experiments due to large differences in allele frequencies in domestic animals and chicken. The effective number of allelic (Ne) frequencies of each microsatellite loci ranged from 2.509 (marker ADL 210) to 13.570 (ADL 136). The mean number of observed allele (Na=10.208) was quite high as compared to the mean number of effective alleles (Ne=6.497) in Mewari chicken.

Table 2: Measures of polymorphism exhibited by microsatellite markers in Mewari chicken

Locus	n	Na	N_e	I	H_o	H_e	μH_e	F_{IS}	PIC	HWE (P-Value)	Sig.
HUJ 2	53	10	4.885	1.903	0.792	0.795	0.803	0.004	0.503	0	***
ADL 23	51	10	4.267	1.755	0.627	0.766	0.773	0.181	0.52	0	***
ADL 136	53	21	13.57	2.782	0.377	0.926	0.935	0.593	0.949	0	***
LEI 146	51	12	7.764	2.261	0.51	0.871	0.88	0.415	0.614	0	***
HUJ 12	55	12	8.438	2.305	0.6	0.881	0.89	0.319	0.91	0	***
ADL 158	48	7	2.738	1.287	0.438	0.635	0.641	0.311	0.839	0	***
ADL 176	44	9	6.185	1.971	0.545	0.838	0.848	0.349	0.877	0	***
ADL 267	46	10	6.904	2.031	0.522	0.855	0.865	0.39	0.686	0	***
MCW 1	55	9	3.965	1.667	0.764	0.748	0.755	-0.021	0.877	0.29	NS
MCW 16	55	9	6.899	2.013	0.509	0.855	0.863	0.405	0.877	0	***
MCW 51	55	9	5.978	1.91	0.582	0.833	0.84	0.301	0.877	0	***
MCW 59	55	12	6.828	2.197	0.927	0.854	0.861	-0.086	0.91	0.04	*
MCW 7	53	6	3.627	1.457	0.434	0.724	0.731	0.401	0.81	0	***
ADL 39	55	6	3.954	1.498	0.182	0.747	0.754	0.757	0.81	0	***
ADL 44	55	5	4.207	1.513	0.527	0.762	0.769	0.308	0.767	0	***
ADL 210	55	3	2.509	1.005	0.655	0.601	0.607	-0.088	0.592	0.40	NS
MCW 11	51	5	3.197	1.287	0.471	0.687	0.694	0.315	0.767	0	***
ADL 102	55	11	5.279	1.946	0.655	0.811	0.818	-0.088	0.9	0	***
ADL 172	51	9	4.222	1.731	0.529	0.763	0.771	0.306	0.814	0.001	***
MCW 43	55	15	10.272	2.477	0.673	0.903	0.911	0.255	0.928	0	***
ADL 34	53	14	10.6	2.456	0.642	0.906	0.914	0.292	0.923	0	***
LEI 65	53	13	9.571	2.4	0.434	0.896	0.904	0.515	0.917	0	***
MCW 135	55	16	12.845	2.63	0.673	0.922	0.931	0.27	0.93	0	***
MCW 312	55	12	7.228	2.193	0.582	0.862	0.87	0.325	0.91	0	***
Mean		10.21	6.497	1.95	0.56	0.81	0.818	0.28	0.81		
SE		0.82	0.63	0.093	0.031	0.018	0.018	0.026	0.026		

(N: Numbers of samples, Na: Numbers of observed alleles, Ne: Numbers of effective alleles, I: Shannon's Information Index, Ho: Observed heterozygosity, Ha: Expected heterozygosity and μHe: Unbiased expected heterozygosity, PIC: Polymorphism Information Content and F_{IS}: Fixation Index, HWE: Hardy-Weinberg Equilibrium, NS =Not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$).

The mean number of observed allele (Na=10.21) in Mewari chicken is higher than those reported by Vij *et al.* (2006) study [13] in Punjab brown chickens (8.38), by Pandey *et al.* (2005) study [14] in Ankleshwar chicken population found in Gujarat (6.6), by Soltan *et al.* (2018) study [15] in Norfa (8.65) and Sinai (8.35) native chicken from Egypt, Ramadan *et al.* (2012) study [16] in six Egyptian strains (6.9), by Abebe *et al.*(2015) study [17] in five Local Swedish Chicken Breeds (4.7 alleles), by Kumar *et al.*(2015) study [18] in Red Jungle Fowl (9.92), in White Leghorn (7.08), in Aseel (8.48), in Red Cornish (7.09), by Faithi *et al.* (2018) study [19] reported 8.6 alleles in Saudi native chicken breeds and by Roh *et al.* (2020) study [20] reported 9.65 alleles in chicken breeds from eight Asian countries. The mean number of observed alleles in Mewari chicken is similar to reported by Rashid *et al.* (2020) study [21] in Indigenous chicken population of Bangladesh (10.7).

Similar allelic range to present study was also reported by Tantia *et al.* (2006) study [22] found in indigenous Indian chicken breeds from 8 to 25 alleles. The present study suggested that the indigenous poultry is highly variable at microsatellite loci.

The mean effective number of alleles reported in present study is similar to reported by Tantia *et al.* (2006) study [22] where 6.27 alleles found in indigenous chicken breeds of India and by Soltan *et al.* (2018) study [15] where Norfa (6.52) and Sinai (6.34) alleles found in native chicken from Egypt. While, the effective number of alleles reported in present study is higher than reported by Pandey *et al.* (2003) study [23] (4.8 in Aseel, 5.27 in Miri and 4.27 in Nicobari) in indigenous chicken breeds of India, by Alipanah *et al.* (2011) study [24] (4.69 in Zabol, 4.50 in Khazak and 5.09 in Dashtiari) in native chicken populations derived from Sistan and Baluchistan province in Iran and by Mukesh *et al.* (2011) study [25] in Red Junglefowl in Northern India (2.628).

Heterozygosity of microsatellite loci

In the present study, the heterozygosity value of each microsatellite locus was calculated in Mewari chicken which are presented in Table 2. As shown in the table, the observed heterozygosity values ranged from 0.182 (ADL 39) to 0.927 (MCW 59) whereas the range of expected heterozygosity was 0.601 (ADL 210) to 0.926 (ADL 136). The average observed heterozygosity and expected heterozygosity value were found to be 0.560 and 0.810, respectively. Thus, it indicates high genetic variation in Mewari chicken.

The mean observed heterozygosity value found in Mewari chicken was similar to reported by Pandey *et al.* (2003) study [23] (0.59, 0.61 and 0.57 respectively, in Aseel, Miri and Nicobari breeds) and the overall heterozygosity value for all the microsatellite loci among these three varieties was observed to be 0.59. The mean observed heterozygosity value in Mewari chicken is also comparable to value reported by Vij *et al.* (2006) study [13] average heterozygosity value in Punjab Brown chicken (0.601).

The mean observed heterozygosity value found in Mewari chicken is lower than reported by Parmar *et al.* (2007) study [26] 0.721, 0.694 and 0.689 values in Jet black, Golden and Pencilled varieties of Kadaknath chicken, respectively. While, the mean observed heterozygosity value found in Mewari chicken is higher than reported by Babar *et al.* (2012) study [27] in Pakistani Aseel chicken (0.3985), by Kumar *et al.* (2015) study [18] reported in Indian Red Jungle Fowl, White Leghorn, Aseel, and Red Cornish (0.457), reported by Bakare *et al.* (2021) study [28] in locally adapted chicken populations in Nigeria (0.396).

The mean effective heterozygosity value were found to be 0.810 in Mewari chicken is higher than reported by Rajkumar *et al.* (2008) study [29] in eight chicken populations from India (0.68), by Kumar *et al.*(2015) study[18] 0.798 (Red Jungle Fowl), 0.695 (White Leghorn), 0.740 (Aseel), 0.696 (Red Cornish), by Sartore *et al.* (2018) study [30] in ISA Brown (0.540) and in Bianca (0.654) two Italian indigenous chicken, by Hariyono *et al.* (2019) study [31] in eight Indonesian local duck populations (0.566) and by Roh *et al.* (2020) study [20] in chicken breeds from eight Asian countries (0.718).

The mean effective heterozygosity value in Mewari chicken is comparable to value reported by Babar *et al.* (2012) study [27] among four varieties of Pakistani Aseel chicken(0.832), by Mukesh *et al.* (2011) study [25] in Red Jungle fowl of Northern India (0.72), by Kumar *et al.* (2015) study [18] in Red Jungle fowl (0.798), Rashid *et al.* (2020) study [21] in five chicken populations of Bangladesh (0.71) and by Sabry *et al.* (2020) study [32] in six Egyptian native chicken strains (0.75). Present finding is also similar to Soltan *et al.* (2016) study [15] reported in Sinai and Norfa chicken from Egypt (0.814) and lower than Bakare *et al.* (2020) study [28] reported in three chicken populations in Nigeria (0.940).

Fixation Index (F_{IS})

The F_{IS} value for all 24 microsatellite loci ranged from -0.088 (ADL 210) to 0.757 (ADL 39) with mean of 0.280 (Table 2) showing considerable level of inbreeding in the Mewari chicken populations. The population is smaller and restricted to central and southern part of Rajasthan reared as backyard chicken. No systematic breeding is practised by keepers. Hence, the same males might have been used over several females for number of generations leading to inbreeding.

The mean F_{IS} value in Mewari chicken is similar observed by Kaya and Yildiz (2008) in Turkish native chicken (mean F_{IS} = 0.301). The mean F_{IS} value in Mewari chicken is higher than reported by Pandey *et al.*(2005) study [33] in Ankleshwar chicken (0.240), by Vij *et al.* (2006) study [13] in Punjab Brown chicken (0.248), by Ding *et al.* (2009) study[34] 0.249, 0.182 and 0.159 in Bian, Jinghai and Youxi Chinese native chicken. While, F_{IS} value in Mewari chicken is much higher than reported by Ramdan *et al.* (2012) study [16] in six Egyptian local chicken strains (0.051), by Chatterjee *et al.* (2015) study [35] in five different breeds/lines in India (-0.18), by Long *et al.* (2017) reported in local chicken breeds of China (0.031) and by Rashid *et al.* (2020) study [21] in five chicken populations of Bangladesh (0.046).

The F_{IS} value in Mewari chicken is lower than reported by Babar *et al.* (2012) study [27] in four Varieties viz. Lakha, Mushki, Mianwali and Peshawari of Pakistani Aseel chicken (0.450), by Mukesh *et al.* (2011) study [25] in Red Jungle fowl of Northern India (0.478), by Soltan *et al.* (2018) study [15] the mean F_{IS} value 0.369 and 0.451 in Norfa and Sinai native chicken of Egypt and by Sabry *et al.* (2020) study [32] reported 0.414 in six Egyptian native chickens.

Polymorphic Information Content (PIC) or Marker Informativeness

In the present study, 19 microsatellite loci showed PIC values of over 0.7, reflecting the prospects of using these loci as candidate genes for the future genetic studies of indigenous chicken breeds. In the present study, the PIC values of all the 24 loci studied ranged from 0.503 (HUJ 2) to 0.949 (ADL 136) with mean 0.813 ± 0.026 indicating markers that were used in Mewari chicken is highly informative.

The PIC values for all the 24 loci in present study is higher than reported by Pandey *et al.* (2003) study [23] mean PIC value 0.64 in (Aseel), 0.66 (Miri) and 0.63 (Nicobari) chicken breeds, by Pandey *et al.* (2005) study [33] mean PIC value 0.672 in Ankleshwar chicken, by Vij *et al*. (2006) study[13] mean PIC value 0.672 in a Punjab Brown chicken, by Parmar *et al.* (2007) study [26] mean PIC value 0.671, 0.699, and 0.617 in Jet black, Golden and Pencilled varieties of Kadaknath breed, respectively, by Babar *et al.* (2012) study [27] mean PIC values to be 0.67, 0.69, 0.71 and 0.65 in individual varieties of Pakistani Aseel chickens. While, the PIC values for all the 24 loci in present study is similar to reported by Soltan *et al.* (2018) study [15] in Norfa and Sinai native chicken of Egypt (0.841), whereas lower than reported by Bakare *et al.* (2020) study [28] in three chicken populations in Nigeria (0.937).

Within Population Genetic Variability

In present study, the Shannon index (I) was found ranged from 1.005 (ADL 210) to 2.782 (ADL 136) with mean of 1.950 which indicated low genetic diversity within population. The value for Shannon index (I) in present study indicated low gene diversity within population in Mewari chicken and value is higher than reported by Pandey *et al.* (2005) study [33] (1.400 in Ankleshwar chicken), by Babar *et al.* (2012) study [27] mean value of Shannon index to be 1.442, 1.538, 1.594 and 1.371 in Lakha, Mushki, Mianwali, Peshwari varieties of Pakistani Aseel respectively. The value for Shannon index (I) in present study is indicating less genetic diversity within population in Mewari chicken is comparable to observed by Mukesh *et al.* (2011) study [25] mean Shannon index value 1.685 in Red Jungle fowl in Northern India.

Hardy-Weinberg Equilibrium

The deviation from the Hardy-Weinberg Equilibrium can be attributed to non-random mating among the individuals of the population and/or due to selection. Exact test for deviations from Hardy-Weinberg equilibrium (HWE) was performed using GeneAlEx version 6.503 (Table: 2). Only microsatellite loci MCW1 and ADL 210 were found to be in HWE. Rest of the microsatellite loci (22) deviated significantly from Hardy-Weinberg equilibrium, which may be due to inbreeding because of small breeding tract and sampling from restricted area.

Similar finding reported by Cuc *et al.* (2006) study [36] only two loci from total 29 loci, deviated from Hardy-Weinberg equilibrium, with only one locus deviating from HWE in all populations, reported by Soltan *et al.* (2018) study [15] that the Norfa chicken population showed deviation from HWE at 16 out of the 20 investigated loci, by Hariyono *et al.* (2019) study [31] observed that seven out of eight populations of local duck of Indonesia showed a departure from Hardy-Weinberg Equilibrium, by Vij *et al.* (2006) study [13] revealed 15 loci deviated from Hardy-Weinberg equilibrium from 26 microsatellite loci in Punjab Brown chicken, by Pandey *et al.* (2005) study [33] reported 14 out of total 25 loci in Ankleshwar chicken showed significant deviations from Hardy Weinberg Equilibrium, Chatterjee *et al.* (2010) study [37] observed four loci from 14 loci deviated from Hardy-Weinberg Equilibrium and remaining markers found to be in equilibrium in White Leghorn chicken.

Conclusions

In the present study, the genetic diversity of indigenous chicken Mewari of southern and central Rajasthan was evaluated using 24 microsatellite markers. The total number of alleles ranged from 3 to 21 with mean number of alleles 10.21 and an effective number of alleles (Ne) ranged from 2.509 to 13.570 with mean effective numbers of allele 6.497 in Mewari chicken. The observed heterozygosity values ranged from 0.182 to 0.927 whereas the range of expected heterozygosity was 0.601 to 0.926. The average observed heterozygosity and expected heterozygosity value were found to be 0.560 and 0.810, respectively. The test for genetic equilibrium indicated that 22, microsatellite loci deviated significantly from Hardy-Weinberg Equilibrium from a total of 24 microsatellite loci in Mewari chicken, might be due to selection operating at linked loci, inbreeding due to small breeding population, sampling from limited area causing relatedness.

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