

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(7): 1148-1155
www.biochemjournal.com
Received: 03-06-2026
Accepted: 04-07-2026

Pradipkumar Parmar
Department of Animal
Genetics & Breeding, College of
Veterinary Science & A. H.,
Kamdhenu University, Anand,
Gujarat, India

Ashish Patel
Department of Animal
Genetics & Breeding, College of
Veterinary Science & A. H.,
Kamdhenu University, Anand,
Gujarat, India

Dhruval Bhatt
Department of Animal
Genetics & Breeding, College of
Veterinary Science & A. H.,
Kamdhenu University, Anand,
Gujarat, India

Prakash Koringa
Department of Animal
Genetics & Breeding, College of
Veterinary Science & A. H.,
Kamdhenu University, Anand,
Gujarat, India

RS Joshi
Department of Animal
Genetics & Breeding, College of
Veterinary Science & A. H.,
Kamdhenu University, Anand,
Gujarat, India

Corresponding Author:
Pradipkumar Parmar
Department of Animal
Genetics & Breeding, College of
Veterinary Science & A. H.,
Kamdhenu University, Anand,
Gujarat, India

Genetic characterization of Mewari chicken based on microsatellite markers

Pradipkumar Parmar, Ashish Patel, Dhruval Bhatt, Prakash Koringa and RS Joshi

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i7o.9353>

Abstract

The present investigation aimed to assess the genetic diversity of the indigenous Mewari chicken population inhabiting the southern and central regions of Rajasthan using 24 microsatellite markers organized into eight multiplex PCR panels. A total of 245 alleles were detected across all loci, with the number of alleles per locus ranging from 3 at ADL 210 to 21 at ADL 136, yielding an average of 10.21 alleles per locus. The mean observed heterozygosity (H_o) and expected heterozygosity (H_e) were estimated at 0.560 and 0.810, respectively. Observed heterozygosity varied between 0.182 (ADL 39) and 0.927 (MCW 59), whereas expected heterozygosity ranged from 0.601 (ADL 210) to 0.926 (ADL 136). The fixation index (FIS) values recorded for the 24 microsatellite loci extended from -0.088 (ADL 210) to 0.757 (ADL 39), with an overall mean of 0.280. The polymorphic information content (PIC) of the markers ranged from 0.503 (HUI 2) to 0.949 (ADL 136), averaging 0.813, indicating that most loci were highly informative. Hardy-Weinberg equilibrium analysis revealed that 22 of the 24 microsatellite loci showed significant deviation from equilibrium expectations. The substantial allelic diversity together with the relatively high heterozygosity values demonstrates considerable genetic variation within the Mewari chicken population, highlighting its rich genetic resource and importance as an indigenous breed of southern and central Rajasthan.

Keywords: Microsatellite, genetic characterization, Mewari chicken, heterozygosity

Introduction

The domestic chicken is believed to have originated primarily from the Red Jungle Fowl (*Gallus gallus*) through a monophyletic evolutionary pathway, although evidence also suggests contributions from several wild species of the genus *Gallus* belonging to the family Phasianidae. These include the Grey Jungle Fowl (*Gallus sonneratii*) native to southwestern India, the Ceylon Jungle Fowl (*Gallus lafayetii*) of Sri Lanka, and the Green Jungle Fowl (*Gallus varius*), which is indigenous to Java and nearby islands [1]. Indigenous and locally adapted chicken breeds represent an invaluable component of global poultry genetic resources, as they have evolved under diverse environmental and geographical conditions, thereby contributing substantially to the genetic diversity observed in poultry populations worldwide [2].

Indigenous chickens constitute an important source of livelihood and nutrition for rural and tribal households in India owing to their ability to thrive under a wide range of agro-climatic conditions [3]. Backyard poultry production is an integral component of the traditional farming system, contributing not only to household income and food security but also to the social and cultural fabric of rural communities. Native chickens possess strong scavenging ability and natural brooding behaviour, enabling them to survive with minimal management inputs. Continuous natural selection under free-range conditions has enhanced their adaptability, making them more tolerant to environmental stress and more resistant to infections caused by bacteria, protozoa, and various internal and external parasites. Consequently, indigenous chickens generally exhibit better adaptability and survivability than commercial hybrid strains under village production systems [5].

Village chickens exhibit a wide range of plumage colours, including brown, black, yellow, and various mixed colour patterns. Such colour variation is considered advantageous, as it provides effective camouflage against natural predators, particularly birds of prey.

These indigenous birds are highly vigilant and possess relatively long, sturdy legs that facilitate rapid escape from predators. In addition, hens retain strong maternal instincts, naturally incubating their eggs and rearing their chicks without external intervention. These behavioural traits enable village chickens to reproduce efficiently and maintain their populations under traditional free-range production systems with minimal human assistance [6].

Genetic diversity is a fundamental resource for the sustainable improvement and conservation of livestock breeds, as it provides the variation necessary for adaptation to evolving environmental conditions and production requirements. Effective conservation and long-term management of Animal Genetic Resources (AnGR) depend on a thorough understanding of their evolutionary background, domestication history, and genetic composition [7]. In many developing countries, indigenous chicken populations are increasingly threatened by genetic erosion resulting from indiscriminate crossbreeding and inbreeding, which may lead to the loss of valuable adaptive traits and unique genetic characteristics [8]. Having evolved over centuries under diverse ecological conditions and traditional management systems, indigenous chicken breeds possess a broad reservoir of genetic variation. This rich genetic diversity represents an important resource for future breeding programmes, conservation efforts, and genetic research.

Microsatellites, also referred to as simple sequence repeats (SSRs) or short tandem repeats (STRs), are short DNA sequences consisting of repeated motifs of one to six nucleotides that are widely distributed throughout eukaryotic genomes. Since their discovery in 1984, these markers have become invaluable tools in molecular genetics because of their high level of polymorphism, co-dominant inheritance, and broad genomic distribution. Their reproducibility, ease of amplification, and ability to detect genetic variation make them particularly suitable for high-resolution genetic mapping and diversity studies. Recent advances in DNA sequencing and genotyping technologies have further improved the speed and efficiency of microsatellite analysis, allowing the simultaneous

genotyping of large numbers of samples within a relatively short period. Consequently, microsatellite markers have been extensively applied in poultry genetics for the characterization, conservation, and improvement of economically important avian species, including chickens, ducks, quails, geese, turkeys, and other domesticated birds [10].

Indian indigenous chickens exhibit considerable phenotypic diversity, reflecting centuries of natural selection and adaptation to diverse agro-ecological conditions under traditional smallholder production systems. Although numerous microsatellite markers have been developed for assessing genetic diversity, relatively few studies have employed these markers to characterize the genetic variability of native chicken ecotypes in India. Despite their economic, ecological, and socio-cultural importance, particularly in rural and tribal communities, comprehensive information on the genetic architecture of these populations including their genetic diversity, population relationships, productive performance, adaptive potential, and disease resistance remains limited [11]. Therefore, the present investigation was undertaken to characterize the genetic diversity of the indigenous Mewari chicken population using microsatellite markers.

Materials and Methods

Blood samples were collected from 60 unrelated Mewari chickens selected randomly from five tehsils Badgaon, Jhadol, Mavli, Nathdwara, and Rajsamand covering the districts of Udaipur, Rajsamand, and Bhilwara in the southern and central regions of Rajasthan. Twenty-four microsatellite markers were chosen for genetic analysis based on their fragment size and fluorescent dye compatibility (Table 1). Amplification of these loci was carried out using multiplex PCR assays, with the markers grouped into seven multiplex panels. Fragment analysis and genotyping were performed on an ABI PRISM 3500 Genetic Analyzer (Applied Biosystems, USA), and allele calling was completed using GeneMapper® Software Version 4.1. The resulting genotypic data were analyzed statistically using GenAlEx Version 6.503.

Table 1: Microsatellite primers used for PCR amplification and their multiplex PCR grouping in Mewari chicken

Sr. No	Markers Name		Sequence (5' 3')	Annealing Temperature (°C)	5' labelling with	Chromosomal Location	Multiplex PCR Set
						Linkage Group	
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	CCACCTCCTTCTCCTGTTC		HEX	10	
4	LEI 146	F	TCAAGCCACCAAAGTGCTTGG	57	FAM	2	
	LEI 146	R	GATCACTCTGCTCATAGCAGT		FAM	C3E6	
5	HUJ 12	F	TAAAAATTATCTTTGAAAAATGCCT	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	ATGGCGCAGAAGGCCAAAGCGATAT	54	HEX	E42	Set-III
	MCW 16	R	TGGCTTCTGAAGCAGTTGCTATGG		FAM	1	
11	MCW 51	F	GGAACAAGCTCTTTCTTCTCCCG	54	HEX	20	
	MCW 51	R	TCATGGAGGTGCTGTGACAAAGAC		FAM	C3	
12	MCW 59	F	AAGTGCCTTTGCTATCCTGATTGG	54	TET	C10,E36	Set-IV
	MCW 59	R	AACTCCTATTGTGCAGCAGCTTAT		FAM	2	
13	MCW 7	F	AGCAAAGAAGTGTTCTCTGTTC	59	FAM	17	
	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	CTGTGGTGTTCGTTAGTTG		HEX	10	
16	ADL 210	F	ACAGGAGGATAGTCACACAT	57	FAM	2	Set-VI
	ADL 210	R	GCCAAAAAGATGAATGAGTA		FAM	C3E6	
17	MCW 11	F	TAAAATTATCTTTGAAAATGCCT	48	FAM	C4E28	
	MCW 11	R	GAGAAACATGTATTTCAATTATTC		FAM	3	
18	ADL 102	F	TTCCACCTTTCTTTTTTATT	48	TET	C3E6	Set-VII
	ADL 102	R	GCTCCACTCCCTTCTAACCC		TET	C1E2	
19	ADL 172	F	CCCTACAACAAAGAGCAGTG	48	FAM	1	
	ADL 172	R	CTATGGAATAAAATGGAAT		TET	15	
20	MCW 43	F	TGACTACTTTGATACGCATGGAGA	57	FAM	12	Set-VIII
	MCW 43	R	CACCAAGTAGACGAAAACACATTT		FAM	11	
21	ADL 34	F	AACCTAAAACTCCTGCTGC	57	HEX	1	
	ADL 34	R	GGGAACCTGTGGGCTGAAAG		FAM	10	
22	LEI 65	F	TGAAACATGTATGGAGTCTCAGCA	57	HEX	E42	Set-IX
	LEI 65	R	GACAGCTAAATGCCAGTTCATGG		FAM	1	
23	MCW135	F	ATATGCTGCAGAGGGCAGTAG	58	HEX	20	
	MCW135	R	CATGTTCTGCATTATTGCTCC		FAM	C3	
24	MCW312	F	TTTGTTCTGGGATTAAGCTTGG	58	TET	C10,E36	Set-X
	MCW312	R	CCTAAATCAGGATGTTTGGAC		FAM	2	

Results and Discussion

Observed and effective number of alleles

A total of 245 alleles were detected across the 24 microsatellite loci analyzed in the Mewari chicken population, demonstrating substantial genetic polymorphism. The observed number of alleles at individual loci ranged from 3 for ADL 210 to 21 for ADL 136, reflecting considerable variation in allelic composition among the loci. The effective number of alleles (N_e), which provides a more realistic estimate of genetic diversity by accounting for allele frequency distribution, was

consistently lower than the observed allele count. This difference is expected because alleles are generally not represented at equal frequencies within populations. In the present study, the effective number of alleles varied between 2.509 (ADL 210) and 13.570 (ADL 136). The average observed number of alleles ($N_a = 10.208$) exceeded the mean effective number of alleles ($N_e = 6.497$), indicating the presence of multiple alleles at several loci but with unequal frequency distributions across the Mewari chicken population.

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

Locus	n	N_a	N_e	I	H_o	H_e	pH_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 μ He: 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 observed number of alleles ($N_a = 10.21$) recorded in the Mewari chicken population was comparatively higher than the values reported for several indigenous and commercial chicken breeds in previous studies. Vij *et al.* (2006) ^[13] reported an average of 8.38 alleles in Punjab Brown chickens, while Pandey *et al.* (2005) ^[14] observed 6.6 alleles in the Ankleshwar chicken population of Gujarat. Similarly, lower mean allele numbers were documented for the Egyptian Norfa (8.65) and Sinai (8.35) chicken populations by Soltan *et al.* (2018) ^[15] and for six Egyptian chicken strains (6.9) by Ramadan *et al.* (2012) ^[16]. Abebe *et al.* (2015) ^[17] reported a mean of 4.7 alleles in five Swedish local chicken breeds, whereas Kumar *et al.* (2015) ^[18] recorded values of 9.92, 7.08, 8.48, and 7.09 for Red Jungle Fowl, White Leghorn, Aseel, and Red Cornish populations, respectively. Likewise, the mean allele number reported for Saudi native chickens by Fathi *et al.* (2018) ^[19] was 8.6, and Roh *et al.* (2020) ^[20] documented a value of 9.65 across chicken breeds from eight Asian countries. In contrast, the mean observed allele number obtained for the Mewari chicken population was closely comparable to that reported for indigenous chickens of Bangladesh (10.7) by Rashid *et al.* (2020) ^[21], indicating a similar extent of genetic diversity.

A comparable range of allelic diversity has also been documented in indigenous Indian chicken breeds, where Tania *et al.* (2006) ^[22] reported between 8 and 25 alleles across microsatellite loci. The relatively high number of alleles observed in the present investigation indicates that the Mewari chicken population possesses substantial genetic variability, demonstrating the effectiveness of microsatellite markers in revealing the rich genetic diversity of this indigenous breed.

The mean effective number of alleles (N_e) observed in the Mewari chicken population was comparable to the values reported for indigenous chicken breeds of India by Tania *et al.* (2006) ^[22], who recorded an average of 6.27, and for the Egyptian Norfa (6.52) and Sinai (6.34) chicken populations described by Soltan *et al.* (2018) ^[15]. However, the mean N_e obtained in the present investigation exceeded those reported for several other native chicken populations. Pandey *et al.* (2003) ^[23] reported effective allele numbers of 4.80, 5.27, and 4.27 in the Aseel, Miri, and Nicobari breeds, respectively. Likewise, Alipanah *et al.* (2011) ^[24] documented values of 4.69, 4.50, and 5.09 for the Zabol, Khazak, and Dashtiari native chicken populations of Iran, while Mukesh *et al.* (2011) ^[25] reported a considerably lower value (2.628) for Red Jungle Fowl from northern India. These comparisons indicate that the Mewari chicken population possesses a relatively high level of effective genetic diversity compared with several other indigenous chicken populations.

Heterozygosity of microsatellite loci

The observed and expected heterozygosity values for each of the 24 microsatellite loci in the Mewari chicken population are presented in Table 4.2. The observed heterozygosity (H_o) varied from 0.182 at the ADL 39 locus to 0.927 at MCW 59, whereas the expected heterozygosity (H_e) ranged between 0.601 for ADL 210 and 0.926 for ADL 136. The mean values of observed and expected heterozygosity were 0.560 and 0.810, respectively. The relatively high heterozygosity estimates obtained in the present study indicate the existence of considerable genetic diversity within the Mewari chicken population.

The mean observed heterozygosity ($H_o = 0.560$) estimated for the Mewari chicken population was consistent with the findings of several earlier investigations on indigenous chicken breeds. Pandey *et al.* (2003) ^[23] reported observed heterozygosity values of 0.59, 0.61, and 0.57 for the Aseel, Miri, and Nicobari breeds, respectively, with an overall mean heterozygosity of 0.59 across these populations. Likewise, Vij *et al.* (2006) ^[13] documented an average observed heterozygosity of 0.601 in Punjab Brown chickens. The close agreement between the present estimate and those reported for these indigenous breeds suggests that the Mewari chicken population possesses a comparable level of within-population genetic variation. Such levels of heterozygosity indicate the maintenance of considerable genetic diversity, which is essential for the long-term adaptability, conservation, and genetic improvement of indigenous chicken germplasm.

The mean observed heterozygosity ($H_o = 0.560$) recorded in the present investigation was comparable with values reported for several indigenous chicken populations. Pandey *et al.* (2003) ^[23] observed heterozygosity estimates of 0.59, 0.61, and 0.57 in the Aseel, Miri, and Nicobari breeds, respectively, with an overall average of 0.59 across the three breeds. Similarly, Vij *et al.* (2006) ^[13] reported an average observed heterozygosity of 0.601 in the Punjab Brown chicken population. The similarity of these estimates to those obtained in the present study indicates that the Mewari chicken population retains a level of genetic variation comparable to other well-characterized indigenous chicken breeds of India. Moderate to high observed heterozygosity reflects the presence of substantial allelic diversity within the population, which contributes to its adaptive potential and evolutionary resilience. This level of genetic variability is advantageous for the conservation of the breed and provides a valuable foundation for future genetic improvement and sustainable breeding programmes.

The mean expected heterozygosity ($H_e = 0.810$) estimated for the Mewari chicken population in the present study was higher than the values reported for several indigenous and commercial poultry populations. Rajkumar *et al.* (2008) ^[29] reported an average expected heterozygosity of 0.680 across eight Indian chicken populations. Similarly, Kumar *et al.* (2015) ^[18] observed expected heterozygosity values of 0.798 in Red Jungle Fowl, 0.695 in White Leghorn, 0.740 in Aseel, and 0.696 in Red Cornish chickens. Lower estimates were also documented by Sartore *et al.* (2018) ^[30], who reported expected heterozygosity values of 0.540 in ISA

Brown and 0.654 in the Bianca, two Italian chicken populations. Likewise, Hariyono *et al.* (2019) ^[31] recorded an average expected heterozygosity of 0.566 among eight Indonesian local duck populations, while Roh *et al.* (2020) ^[20] reported a mean value of 0.718 in chicken breeds representing eight Asian countries. The comparatively higher expected heterozygosity observed in the present investigation indicates that the Mewari chicken population harbours a rich reservoir of genetic diversity. Such elevated levels of genetic variability are indicative of a broad genetic base and highlight the importance of this indigenous breed as a valuable genetic resource for conservation, selective breeding, and future improvement programmes.

The mean expected heterozygosity ($H_e = 0.810$) estimated for the Mewari chicken population was comparable with values reported for several indigenous chicken populations from different geographical regions. Babar *et al.* (2012) ^[27] reported an expected heterozygosity of 0.832 among four varieties of Pakistani Aseel chickens, while Mukesh *et al.* (2011) ^[25] and Kumar *et al.* (2015) ^[18] recorded values of 0.720 and 0.798, respectively, in Red Jungle Fowl populations of northern India. Similarly, Rashid *et al.* (2020) ^[21] observed an average expected heterozygosity of 0.710 across five indigenous chicken populations of Bangladesh, and Sabry *et al.* (2020) ^[32] reported a value of 0.750 in six Egyptian native chicken strains. The present estimate was also in close agreement with the findings of Soltan *et al.* (2018) ^[15], who reported an expected heterozygosity of 0.814 in the Sinai and Norfa chicken populations of Egypt. However, the value obtained in the present investigation was lower than the exceptionally high expected heterozygosity (0.940) reported by Bakare *et al.* (2020) ^[28] in three Nigerian indigenous chicken populations. Overall, these comparisons indicate that the Mewari chicken population possesses a high level of expected heterozygosity comparable to many well-characterized indigenous chicken breeds worldwide, reflecting a broad genetic base and emphasizing its significance as an important genetic resource for conservation and genetic improvement programmes.

Fixation index (F_{IS})

The inbreeding coefficient (FIS) estimated across the 24 microsatellite loci in the Mewari chicken population is presented in Table 4.2. The FIS values varied from -0.088 at the ADL 210 locus to 0.757 at the ADL 39 locus, with an overall mean value of 0.280. The negative FIS value observed at the ADL 210 locus indicates a slight excess of heterozygotes at that marker, whereas the positive values recorded for most loci suggest varying degrees of heterozygote deficiency within the population. The positive mean FIS value indicates a moderate level of inbreeding in the Mewari chicken population.

The observed pattern of inbreeding may be attributed to the management and breeding practices followed in the native breeding tract of the Mewari chicken. This breed is primarily distributed in the central and southern regions of Rajasthan, where it is maintained under traditional backyard production systems. Since organized breeding programmes and pedigree-based selection are generally absent, mating occurs naturally without planned genetic management. Under such conditions, the repeated use of a limited number of breeding males across several females over successive generations is likely to increase the level of relatedness

among individuals, resulting in heterozygote deficiency and a corresponding increase in the inbreeding coefficient. These findings emphasize the need for scientifically planned breeding and conservation strategies to preserve the genetic variability of the Mewari chicken while minimizing further inbreeding.

The mean inbreeding coefficient ($F_{IS} = 0.280$) estimated for the Mewari chicken population was comparable to the value reported by Kaya and Yildiz (2008), who observed a mean FIS of 0.301 in Turkish native chickens. However, the mean FIS obtained in the present study was higher than those reported for several indigenous chicken populations. Pandey *et al.* (2005) ^[33] reported an average FIS of 0.240 in Ankleshwar chickens, while Vij *et al.* (2006) ^[13] recorded a value of 0.248 in Punjab Brown chickens. Similarly, Ding *et al.* (2009) ^[34] reported FIS values of 0.249, 0.182, and 0.159 in the Bian, Jinghai, and Youxi Chinese native chicken breeds, respectively. In contrast, substantially lower FIS values have been documented in several other poultry populations. Ramdan *et al.* (2012) ^[16] reported a mean FIS of 0.051 in six Egyptian local chicken strains, whereas Chatterjee *et al.* (2015) ^[35] observed a negative value (-0.180) among five Indian chicken breeds and lines, indicating an excess of heterozygotes. Likewise, Long *et al.* (2017) reported a low FIS value of 0.031 in Chinese local chicken breeds, and Rashid *et al.* (2020) ^[21] reported an average FIS of 0.046 in five indigenous chicken populations of Bangladesh.

The comparatively higher FIS value observed in the present investigation suggests a greater degree of heterozygote deficiency and inbreeding in the Mewari chicken population than in many previously studied indigenous breeds. This pattern is likely a consequence of the breed's limited population size, restricted geographical distribution, and the absence of organized breeding programmes, which may have resulted in repeated mating among related individuals over successive generations. These findings underscore the importance of implementing structured breeding and conservation programmes to maintain genetic diversity, reduce inbreeding, and ensure the long-term sustainability of the Mewari chicken genetic resource.

The mean inbreeding coefficient ($F_{IS} = 0.280$) estimated for the Mewari chicken population was lower than the values reported for several indigenous chicken populations from other countries. Babar *et al.* (2012) ^[27] reported a mean FIS of 0.450 among four Pakistani Aseel varieties, namely Lakha, Mushki, Mianwali, and Peshawari, indicating a higher level of inbreeding than that observed in the present study. Similarly, Mukesh *et al.* (2011) ^[25] recorded a FIS value of 0.478 in Red Jungle Fowl populations of northern India. Soltan *et al.* (2018) ^[15] also reported comparatively higher mean FIS values of 0.369 and 0.451 in the Norfa and Sinai native chicken populations of Egypt, respectively. Likewise, Sabry *et al.* (2020) ^[32] observed a mean FIS of 0.414 among six Egyptian native chicken strains. These comparisons indicate that, although the Mewari chicken population exhibits evidence of inbreeding, the extent of inbreeding is relatively lower than that reported for several other indigenous chicken populations. The comparatively lower FIS value suggests that the Mewari chicken has retained a moderate level of genetic diversity despite its restricted geographical distribution and backyard production system. Nevertheless, the positive FIS estimate highlights the need for appropriate breeding strategies and

conservation measures to minimize further inbreeding and preserve the long-term genetic health of this valuable indigenous genetic resource.

Polymorphic information content (PIC) or marker informativeness

The polymorphic information content (PIC) values calculated for the 24 microsatellite loci analyzed in the Mewari chicken population are presented in Table 4.2. The PIC values ranged from 0.503 at the HUI 2 locus to 0.949 at the ADL 136 locus, with an overall mean of 0.813 ± 0.026 . According to the classification proposed by Botstein *et al.* (1980), markers with PIC values greater than 0.50 are considered highly informative. In the present investigation, all the microsatellite loci exhibited PIC values above 0.50, while 19 of the 24 loci showed PIC values exceeding 0.70, indicating a high degree of polymorphism and excellent discriminatory power. The high PIC values obtained in this study demonstrate that the selected microsatellite markers are highly informative for assessing genetic diversity and population structure in the Mewari chicken. Furthermore, these markers can serve as reliable molecular tools for future studies involving genetic characterization, conservation genetics, parentage verification, population differentiation, and marker-assisted breeding of indigenous chicken breeds. The overall high PIC values also reflect the substantial allelic diversity present within the Mewari chicken population, further supporting the existence of a broad genetic base in this indigenous breed.

The mean polymorphic information content ($PIC = 0.813 \pm 0.026$) obtained in the present study was higher than the values reported for several indigenous chicken populations. Pandey *et al.* (2003) [23] reported mean PIC values of 0.640, 0.660, and 0.630 for the Aseel, Miri, and Nicobari breeds, respectively. Similarly, Pandey *et al.* (2005) [33] recorded a mean PIC value of 0.672 in Ankleshwar chickens, while Vij *et al.* (2006) [13] reported an identical value (0.672) in Punjab Brown chickens. Parmar *et al.* (2007) [26] observed mean PIC values of 0.671, 0.699, and 0.617 in the Jet Black, Golden, and Pencilled varieties of the Kadaknath breed, respectively. Likewise, Babar *et al.* (2012) [27] reported mean PIC values of 0.670, 0.690, 0.710, and 0.650 among four Pakistani Aseel varieties. The comparatively higher PIC values obtained in the present investigation indicate that the microsatellite markers employed were more informative and revealed a greater degree of genetic polymorphism in the Mewari chicken population than those reported in these earlier studies.

The mean PIC value observed in the present study was comparable to the estimate reported by Soltan *et al.* (2018) [15], who recorded a mean PIC value of 0.841 in the Norfa and Sinai native chicken populations of Egypt. However, it was lower than the exceptionally high mean PIC value of 0.937 reported by Bakare *et al.* (2020) [28] in three indigenous chicken populations of Nigeria. Overall, the high PIC values obtained in the present investigation confirm that the selected microsatellite markers possess excellent discriminatory power and are highly suitable for evaluating genetic diversity, population structure, conservation genetics, and future breeding programmes in the Mewari chicken population.

Within population genetic variability

The Shannon's information index (I) estimated for the 24 microsatellite loci in the Mewari chicken population ranged

from 1.005 at the ADL 210 locus to 2.782 at the ADL 136 locus, with a mean value of 1.950. Shannon's information index is a widely used measure of genetic diversity that accounts for both the number of alleles and their distribution within a population. The relatively high mean value obtained in the present study indicates the presence of substantial allelic richness and genetic variation in the Mewari chicken population.

The mean Shannon's information index recorded in the present investigation was higher than the values reported for several indigenous chicken populations. Pandey *et al.* (2005) [33] reported a mean value of 1.400 in Ankleshwar chickens, while Babar *et al.* (2012) [27] observed mean Shannon's information index values of 1.442, 1.538, 1.594, and 1.371 in the Lakha, Mushki, Mianwali, and Peshawari varieties of Pakistani Aseel chickens, respectively. The estimate obtained in the present study was also higher than the mean value of 1.685 reported by Mukesh *et al.* (2011) [25] for Red Jungle Fowl from northern India. These comparisons suggest that the Mewari chicken population possesses a relatively high level of genetic diversity and allelic richness compared with several other indigenous chicken populations. The elevated Shannon's information index further supports the findings obtained from heterozygosity, allele number, and polymorphic information content analyses, confirming that the Mewari chicken represents a genetically diverse indigenous breed with considerable potential for conservation and future genetic improvement programmes.

Hardy-Weinberg Equilibrium

The Shannon's information index (I) estimated across the 24 microsatellite loci in the Mewari chicken population ranged from 1.005 at the ADL 210 locus to 2.782 at the ADL 136 locus, with an overall mean value of 1.950. Shannon's information index is a robust measure of genetic diversity that incorporates both allelic richness and the relative frequency of alleles within a population. The relatively high mean value obtained in the present investigation reflects the presence of abundant genetic variation and a broad allelic base in the Mewari chicken population. These findings are consistent with the high levels of heterozygosity, allele diversity, and polymorphic information content observed in the present study, further demonstrating the rich genetic constitution of this indigenous breed.

The mean Shannon's information index recorded in the present investigation exceeded the values reported for several indigenous chicken populations. Pandey *et al.* (2005) [33] reported a mean Shannon's information index of 1.400 in Ankleshwar chickens, while Babar *et al.* (2012) [27] documented mean values of 1.442, 1.538, 1.594, and 1.371 in the Lakha, Mushki, Mianwali, and Peshawari varieties of Pakistani Aseel chickens, respectively. Similarly, Mukesh *et al.* (2011) [25] reported a mean Shannon's information index of 1.685 in Red Jungle Fowl from northern India. The comparatively higher Shannon's information index obtained in the present study indicates that the Mewari chicken population harbours greater allelic diversity than these previously studied populations. Collectively, these observations suggest that the Mewari chicken possesses a broad genetic base, making it an important indigenous genetic resource with considerable potential for conservation, sustainable utilization, and future genetic improvement programmes.

Conclusion

The present study assessed the genetic diversity of the indigenous Mewari chicken population of central and southern Rajasthan using 24 FAO-recommended microsatellite markers. A total of 245 alleles were detected across all loci, with the number of alleles per locus varying from 3 to 21 and an average of 10.21 alleles. The effective number of alleles (N_e) ranged from 2.509 to 13.570, with a mean value of 6.497, indicating substantial allelic diversity within the population. The observed heterozygosity (H_o) varied between 0.182 and 0.927, while the expected heterozygosity (H_e) ranged from 0.601 to 0.926, with mean values of 0.560 and 0.810, respectively. The comparatively high expected heterozygosity and allelic richness suggest that the Mewari chicken population possesses considerable genetic variability and a broad genetic base.

Analysis of Hardy-Weinberg equilibrium (HWE) revealed that 22 of the 24 microsatellite loci showed significant deviations from equilibrium. Such deviations are likely attributable to a combination of biological and management factors, including selection acting on linked genomic regions, the relatively small effective breeding population, non-random mating, and inbreeding resulting from the repeated use of a limited number of breeding males under traditional backyard production systems. In addition, sampling from a geographically restricted breeding tract may have increased the level of relatedness among sampled individuals. Collectively, these findings indicate that although the Mewari chicken population retains a high level of genetic diversity, the observed heterozygote deficiency and departure from Hardy-Weinberg equilibrium highlight the need for scientifically planned breeding and conservation programmes to maintain its genetic variability and minimize further inbreeding.

References

- Laval G, SanCristobal M, Chevalet C. Measuring genetic distances between breeds: use of some distances in various short term evolution models. *Genetics Selection Evolution*. 2002;34(4):1-27.
- Malvika S, Ghosh PR, Dhar B, Devi NN, Paul R, Halder A, *et al*. Genetic status of indigenous poultry (red jungle fowl) from India. *Gene*. 2019;705:77-81.
- Khan AG. Indigenous breeds, crossbreeds and synthetic hybrids with modified genetic and economic profiles for rural family and small-scale poultry farming in India. *World's Poultry Science Journal*. 2008;64(3):405-415.
- Minga U, Msoffe PL, Gwakisa PS. Biodiversity (variation) in disease resistance and in pathogens within a rural chicken population. In: *Proceedings of the 22nd World's Poultry Congress*; 2004 Jun 8-12.
- Besbes B, Tixier-Boichard M, Hoffmann I, Jain GL. Future trends for poultry genetic resources. In: *Proceedings of the International Conference on Poultry in the 21st Century: Avian Influenza and Beyond*; 2007. p. 5-7.
- Felius M, Theunissen B, Lenstra JA. Conservation of cattle genetic resources: the role of breeds. *The Journal of Agricultural Science*. 2015;153(1):152-162.
- Food and Agriculture Organization of the United Nations. Molecular genetic characterization of animal genetic resources. *Animal Production and Health Guidelines No. 9*. Rome (Italy): FAO; 2011. p. 84.
- Gholizadeh M, Mianji GR. Use of microsatellite markers in poultry research. *International Journal of Poultry Science*. 2007;6(2):145-153.
- Rudresh B. Molecular characterization of indigenous chicken in different geographical locations of Karnataka [doctoral dissertation]. Bidar: Karnataka Veterinary, Animal and Fisheries Sciences University; 2014. Available from: <http://krishikosh.egranth.ac.in/handle/1/67507>
- Vij PK, Tania MS, Vijh RK. Characterization of Punjab brown chicken. *Animal Genetic Resources*. 2006;39:65-76.
- Pandey AK, Kumar D, Sharma R, Sharma U, Vijh RK, Ahlawat SPS. Population structure and genetic bottleneck analysis of Ankleshwar poultry breed by microsatellite markers. *Asian-Australasian Journal of Animal Sciences*. 2005;18(7):915-921.
- Soltan M, Farrag S, Enab A, Abou-Elewa E, El-Safty S, Abushady A. Sinai and Norfa chicken diversity revealed by microsatellite markers. *South African Journal of Animal Science*. 2018;48(2):307-315.
- Ramadan S, Kayang BB, Inoue E, Nirasawa K, Hayakawa H, Ito SI, *et al*. Evaluation of genetic diversity and conservation priorities for Egyptian chickens. 2012.
- Abebe AS, Mikko S, Johansson AM. Genetic diversity of five local Swedish chicken breeds detected by microsatellite markers. *PLoS One*. 2015;10(4):1-13.
- Kumar V, Shukla SK, Mathew J, Sharma D. Genetic diversity and population structure analysis between Indian red jungle fowl and domestic chicken using microsatellite markers. *Animal Biotechnology*. 2015;26(3):201-210.
- Fathi MM, Al-Homidan I, Motawei MI, Abou-Emera OK, El-Zarei MF. Evaluation of genetic diversity of Saudi native chicken populations using microsatellite markers. *Poultry Science*. 2017;96(3):530-536.
- Roh HJ, Kim SC, Cho CY, Lee J, Jeon D, Kim DK, *et al*. Estimating genetic diversity and population structure of 22 chicken breeds in Asia using microsatellite markers. *Asian-Australasian Journal of Animal Sciences*. 2020;33(12):1896.
- Rashid MA, Manjula P, Faruque S, Bhuiyan AFH, Seo D, Alam J, *et al*. Genetic diversity and population structure of indigenous chicken of Bangladesh using microsatellite markers. *Asian-Australasian Journal of Animal Sciences*. 2020;33(11):1732.
- Tania MS, Vijh RK, Kumar SB, Mishra B, Ahlawat SPS. Genetic diversity analysis of chicken breeds of India. *The Indian Journal of Animal Sciences*. 2006;76(12).
- Pandey AK, Tania MS, Kumar D, Mishra B, Chaudhary P, Vijh RK. Microsatellite analysis of three poultry breeds of India. *Asian-Australasian Journal of Animal Sciences*. 2003;15(11):1536-1542.
- Alipanah M, Torkamanzehi A, Amiry Z, Rabani F. Study of genetic diversity of Dashtiari, Khazak and Zabol chickens using microsatellite markers. *Trakia Journal of Sciences*. 2011;9:76-81.
- Mukesh R, Kalsi S, Mandhan RP, Sathyakumar S. Genetic diversity studies of red jungle fowl across its distribution range in Northern India. *Asian Journal of Biotechnology*. 2011;3:293-301.

23. Parmar SNS, Tolenkhomba TC, Thakur MS, Joshi CG, Rank DN, Solanki JV, *et al.* Analysis of genetic relationship among three varieties of indigenous Kadaknath breed using 25 chicken microsatellite markers. 2007.
24. Babar ME, Nadeem A, Hussain T, Wajid A, Shah SA, Iqbal A, *et al.* Microsatellite marker-based genetic diversity among four varieties of Pakistani Aseel chicken. Pakistan Veterinary Journal. 2012;32(2):237-241.
25. Bakare IO, Ilori BM, Wheto M, Egbeyale LT, Sanda AJ, Olowofeso O. Genetic diversity and gene flow among three chicken populations in Nigeria using microsatellite markers. Agriculturae Conspectus Scientificus. 2021;86(2):173-181.
26. Rajkumar U, Gupta BR, Reddy AR. Genomic heterogeneity of chicken populations in India. Asian-Australasian Journal of Animal Sciences. 2008;21(12):1710-1720.
27. Sartore S, Sacchi P, Soglia D, Maione S, Schiavone A, De Marco M, *et al.* Genetic variability of two Italian indigenous chicken breeds inferred from microsatellite marker analysis. British Poultry Science. 2016;57(4):435-443.
28. Hariyono DNH, Maharani D, Cho S, Manjula P, Seo D, Choi N, *et al.* Genetic diversity and phylogenetic relationship analyzed by microsatellite markers in eight Indonesian local duck populations. Asian-Australasian Journal of Animal Sciences. 2019;32(1):31.
29. Sabry A, Ramadan S, Hassan MM, Mohamed AA, Mohammedin A, Inoue-Murayama M. Assessment of genetic diversity among Egyptian and Saudi chicken ecotypes and local Egyptian chicken breeds using microsatellite markers. Journal of Environmental Biology. 2021;42(1):33-39.
30. Pandey AK, Kumar D, Sharma R, Sharma U, Viji RK, Ahlawat SPS. Population structure and genetic bottleneck analysis of Ankleshwar poultry breed by microsatellite markers. Asian-Australasian Journal of Animal Sciences. 2005;18(7):915-921.
31. Ding FX, Zhang GX, Wang JY, Li Y, Zhang LJ, Wei Y, *et al.* Genetic diversity of a Chinese native chicken breed, Bian chicken, based on twenty-nine microsatellite markers. Asian-Australasian Journal of Animal Sciences. 2009;23(2):154-161.
32. Chatterjee RN, Bhattacharya TK, Dange M, Dushyanth K, Niranjana M, Reddy BLN, *et al.* Genetic heterogeneity among various indigenous and other chicken populations with microsatellite markers. Journal of Applied Animal Research. 2015;43(3):266-271.
33. Cuc NTK, Muchadeyi FC, Baulain U, Eding H, Weigend S, Wollny CBA. An assessment of genetic diversity of Vietnamese H'mong chickens. International Journal of Poultry Science. 2006;5(10):912-920.
34. Chatterjee RN, Bhattacharya TK, Dange M, Rajkumar U. Assessment of genetic relatedness of crossbred chicken populations using microsatellite markers. Biochemical Genetics. 2010;48(9-10):727-736.