RESEARCH ARTICLE

Structure Diversity Assessment of Native Chicken Population of North Gujarat by Hardy Weinberg Equilibrium Test and Bottleneck Analysis

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Abstract

Genetic characterization evaluates the population's genetic homogeneity, heterogeneity, inbreeding or introgression. The evolutionary history of a species or breed and/or population is revealed through the phylogenetic relationships between populations based on microsatellite analysis. Even the status of the breeds in the specified geographic area can be monitored to prioritize the breed's conservation. Based on this a *desi*/native chicken population reared by tribal communities from North Gujarat were characterized by 25 microsatellite markers on 60 chicken blood samples. The microsatellites were amplified in seven multiplex panels using fluorescently labelled primers and genotyped on genetic analyzer system. In results, only five microsatellites were found to be in Hardy-Weinberg Equilibrium (HWE). Rest of the microsatellite loci deviated significantly ($p<0.01$) from HWE. The Bottleneck program test for departure from mutation-drift equilibrium based on heterozygosity deficit or excess revealed that this chicken population did not suffer from bottleneck in recent past.

Key words: Bottleneck analysis, Genetic characterization, Hardy-Weinberg equilibrium, Native chicken of Gujarat.

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Introduction

Rearing of indigenous chicken plays an important role in rural and tribal family poultry keeping in India mainly due to their adaptability to different agroclimatic conditions (Khan, 2008). Back yard poultry farming is a part and parcel of typical rural/tribal household, touching social, cultural and economic aspects in India. Local and/or indigenous chicken breeds, which are as a result of centuries of adaptation, domestication and breeding, represent an important source of genes for future breeding and research purposes and are considered a huge treasure of variable genotypes. Tribal people of Banaskantha, Sabarkantha, Arvalli, and Mahisagar districts of North Gujarat are raising native chickens as a form of household poultry farming to generate revenue. Phylogenetic relationships of populations based on genetic analysis unravel the evolutionary history of the breeds/populations of a species. Through this, we can prioritize the breeds for conservation using molecular data and monitor its status in the defined geographical region (ICAR-NBAGR, 2016).

Microsatellites are the marker of choice for biodiversity evaluation owing to their unique characteristics and ease of applications. By looking at the variation of microsatellites, inferences can be made about genetic distance estimates and relationships, characterization of different breeds or strains within a species, assessing population structure, genetic drift, parentage assessment etc. (Vignal *et al.,* 2002). There is potential for distinct population of chicken having unique characters away from the known breeds of Gujarat and neighboring states. Hence, this study was undertaken to evaluate diversity structure of native chicken population of North Gujarat by HWE test and Bottleneck analysis.

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Materials and Methods

Sixty blood samples were randomly taken from genetically unrelated chickens in three pockets namely Amirgadh, Vadgam, and Danta of Banaskantha district of North Gujarat. The DNA was isolated using standard laboratory protocol with suitable modifications (John *et al*., 1991).

Quality and purity of DNA were checked by 0.8 % agarose gel electrophoresis. Additionally, purity of DNA was also checked by ND-1000 Spectrophotometer (NanoDrop Technologies, Inc. USA). OD at 260 and 280 were taken against the TE buffer.

A total of 25 fluorescent labelled primers used were divided in to seven multiplex primers panels with annealing temperatures (Table 1). Only forward primer of each pair was labelled with one of the three fluorophores, *i.e.,* FAM, HEX and

Table 1: Primers used for PCR amplification of microsatellite loci

Multiplex PCR Panel (Annealing Temperature)	Microsatellite Locus	Sequence (5′ 3′)		Chromosomal Location/ Linkage Group	5′ labelling with	References
Panel I (57 °C)	HUJ-2	F	CATCTCACAGAGCAGCAGTG	17	FAM	Parmar *et al.* (2007)
		R	GAATCCTGGATGTCAAAGCC			
	ADL136	F	TGTCAAGCCCATCGTATCAC	9	HEX	Das *et al.* (2015)
		R	CCACCTCCTTCTCCTGTTCA			
	LEI-146	F	TCAAGCCACCAAAGTGCTTGG	1	TET	Crooijmans *et al.* (1997)
		R	GATCACTCTGCTCATAGCAGT			
	ADL-23	F	CTTCTATCCTGGGCTTCTGA	5	FAM	Parmar *et al.* (2007)
		R	CCTGGCTGTGTATGTGTTGC			
	ADL-158	F	TGGCATGGTTGAGGAATACA	10	HEX	Das *et al.* (2015)
		R	TAGGTGCTGCACTGGAAATC			
Panel II (57 °C)	HUJ-12	F	GTCTCATGCTATGAGAGTGG	8	TET	Parmar *et al.* (2007)
		R	CCTCTGGTTGAATCAGTCTG			
	ADL-267	F	AAACCTCGATCAGGAAGCAT	C3E6	FAM	Das *et al.* (2015)
		R	GTTATTCAAAGCCCCACCAC			
	ADL-176	F	TTGTGGATTCTGGTGGTAGC	2	FAM	Das *et al.* (2015)
		R	TTCTCCCGTAACACTCGTCA			
Panel III (54 °C)	MCW-1	F	TGTCACAGTGGGGTCATGGACA	C4E28	FAM	Parmar *et al.* (2007)
		R	ACACGTCCTGTGTTCACATGCCTGT			
	MCW-16	F	ATGGCGCAGAAGGCAAAGCGATAT	3	FAM	Cuc *et al.* (2006)
		R	TGGCTTCTGAAGCAGTTGCTATGG			
	MCW-51	F	GGAACAAGCTCTTTCTTCTTCCCG	C3E6	TET	Das *et al.* (2015)
		R	TCATGGAGGTGCTGGTACAAAGAC			
	MCW-59	F	AAGTGCCTTTGCTATCCTGATTGG	C1E2	TET	Babar *et al.* (2012)
		R	AACTCCTATTGTGCAGCAGCTTAT			
Panel IV (59 °C)	MCW7	F	AGCAAAGAAGTGTTCTCTGTTCAT	1	FAM	Parmar *et al.* (2007)
		R	ACCCTGCAAACTGGAAGGGTCTCA			
	MCW73	F	TATTTCACCCACGGGGACGAATAC	C33E46	HEX	Parmar *et al.* (2007)
		R	AGGGTGCTGAGAGCTGCCAATGTC			
	MCW49	F	AGCGGCGTTGAGTGAGAGGAGCGA	1	HEX	Chatterjee *et al.* (2010)
		R	TCCCCAACCCGCGGAGAGCGCTAT			
Panel V (57 °C)	ADL-39	F	GCTACAACGCTTCAAACCTG	15	TET	Parmar *et al.* (2007)
		R	ACAAACAAACCAAAAAACCT			
	ADL-44	F	AAGTGGTTTATTGAAGTAGA	12	FAM	Parmar *et al.* (2007)
		R	CTGTGGTGTTGCGTTAGTTG			
	ADL210	F	ACAGGAGGATAGTCACACAT	11	FAM	Das *et al.* (2015)
		R	GCCAAAAAGATGAATGAGTA			
Panel VI (48 °C)	MCW-11	F	TAAAATTTATCTTTGAAAATGCCT	1	HEX	Crooijmans *et al.* (1997)
		R	GAGAAACATGTATTTCCAATTATTC			
	ADL-102	F	TTCCACCTTTCTTTTTTATT	10	FAM	Das *et al.* (2015)
		R	GCTCCACTCCCTTCTAACCC			
	ADL-172	F	CCCTACAACAAAGAGCAGTG	E42	HEX	Das *et al.* (2015)
		R	CTATGGAATAAAATGGAAAT			
Panel VII (57 °C)	MCW-43	F	TGACTACTTTGATACGCATGGAGA	1	FAM	Das *et al.* (2015)
		R	CACCAAGTAGACGAAAACACATTT			
	LEI-65	F	TGAAACATGTATGGAGTCTCAGCA	C3	FAM	Gibbs *et al.* (1997)
		R	GACAGCTAAATGCCAGTTCATGG			
	ADL-34	F	AACCTAAAAACTCCTGCTGC	20	HEX	Parmar *et al.* (2007)
		R	GGGAACCTGTGGGCTGAAAG			
	HUJ-1	F	CCATCCGCTTATACAGAGCACA	E01	TET	Parmar *et al.* (2007)
		R	CCCTTTGTTAACACCTACTGCA			

TET dye phosphoramidites synthesized by Applied Biosystems, USA.

The fragment size and fluorescent dye label of the 25 microsatellite primers were taken into consideration. Twenty-five microsatellite loci were amplified using PCR in seven multiplexed panels. The genotyping was carried out by utilizing the automated DNA sequencer, genetic analyzer ABI PRISM 3500 and Gene Mapper software version 4.1 (Applied Biosystem, USA). The exact test for deviation from Hardy Weinberg Equilibrium was also carried out as implemented in Genepop version 4.2. Additionally, the mutation drift equilibrium test was applied using all the three models of microsatellite evolution using Bottleneck software version 1.2.02 (Piry *et al.,* 1999)

Result and Discussion

Preliminary Analysis of Microsatellite Markers

A total of 240 alleles were found, where observed number of alleles per locus varied from 6 (ADL 172, MCW 43) to 15 (ADL 136). The mean observed numbers of alleles were found to be 9.60. The overall means for observed and expected heterozygosities were 0.531 and 0.771, respectively. Polymorphic Information Content (PIC) value ranged from 0.494 (ADL 158) to 0.883 (ADL 136) with a mean of 0.747. The high number of observed alleles and high heterozygosity indicated presence of high genetic variability in native chicken and selected microsatellites were highly polymorphic as well as proved very useful for breed characterization.

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 the Genepop version 4.2. Microsatellite loci HUJ 2, ADL 23, MCW 49, MCW 43 and HUJ 1 were found to be in HWE. Rest of the microsatellite loci deviated significantly ($p<0.01$) from HWE (Table 2). Thus, native chicken population was found to be deviating at most of the loci studied in the present study, which could be due to selection or inbreeding.

Similar results were found in a study on White Leghorn chicken by Chatterjee *et al.* (2010), where observed four loci from 14 loci deviated from Hardy-Weinberg Equilibrium and remaining markers were found to be in equilibrium. Pandey *et al.* (2005) found 14 out of total 25 loci in Ankleshwar chicken showing significant deviations from HWE. Vij *et al.* (2006) revealed 15 loci deviating from HWE from 26 microsatellite loci in Punjab Brown chicken. Saini *et al.* (2007) reported that 10 microsatellite loci deviated from HWE among two selected strains of White Leghorn, an unselected control line of White Leghorn and two selected strains of Rhode Island Red. Soltan *et al.* (2017) reported that the Norfa chicken population showed deviation from HWE at 16 out of the 20 investigated loci, while the Sinai chicken population of Egpyt showed a deviation at 17 out of the 20 loci. Hariyono *et al.* (2019) observed seven out of eight populations of local duck of Indonesia departing from HWE. Parmar *et al.* (2022) estimated only microsatellite loci MCW1 and ADL 210 to be in HWE. Rest of the microsatellite loci (22) deviated significantly from HWE in Mewari chicken. Qu *et al.* (2006) observed most of the microsatellite loci in all populations of indigenous chicken breeds of China in agreement with HWE, with only one locus (LEI0194) deviating from HWE. Cuc *et al.* (2006) reported only two loci (MCW 123 and LEI 234) from 29 loci, deviating from HWE, and opined that all the village-based populations were not affected by inbreeding.

Bottleneck Analysis

Recent genetic bottleneck was tested using the statistical test based on the difference between allelic diversity and heterozygosity (Piry *et al.,* 1999). The sign test revealed significant heterozygosity excess ($p<0.01$) in Infinite Allele Model (IAM) and Stepwise Mutation Model (SMM), whereas non-significant in Two Phase Model (TPM). The results obtained for the standardized difference test in which the T2 values were found significantly positive under the two mutation models 3.614 (IAM) and 0.386 (TPM) and negative in SMM (-6.759) (Table 3). The standardized difference test revealed significant value for IAM and SMM, but not in TPM model. The Wilcoxon tests revealed significant heterozygosity excess value for IAM, whereas non-significant in SMM and TPM models. The IAM and SMM are the most

Table 2: Hardy-Weinberg-Equilibrium (P value) for different loci

Locus	HWE (P value)	Locus	HWE (P value)	Locus	HWE (P value)
HUJ 2	0.074 (NS)	MCW 16	0.005**	ADL 210	0.000**
ADL 136	0.000**	MCW 51	0.000**	MCW 11	0.000**
LEI 146	0.000**	MCW 59	0.001**	ADL 102	0.000**
ADL 23	0.130 NS	MCW 7	0.000**	ADL 172	0.000**
ADL 158	0.009**	MCW 73	0.000**	MCW 43	0.073 NS
HUJ 12	0.000**	MCW 49	0.245 NS	LEI 65	0.003**
ADL 267	0.000**	ADL 39	0.000**	ADL 34	0.008**
ADL 176	0.000**	ADL 44	0.000**	HUJ 1	0.411 NS
MCW 1	0.000**	**p<0.01, NS: Non-significant.			

suitable models for microsatellite evolution. Thus, based on SMM model and Wilcoxon test native chicken population did not experience any genetic bottleneck in recent past. The mode shift exhibited no distortion of allelic frequency and form normal L-shaped distribution (Fig. 1).

Similar results were obtained by Vij *et al.* (2006) with normal L-shaped distribution and thus, concluded that the Punjab Brown breed had not experienced any recent genetic bottleneck. Pandey *et al.* (2005) observed lack of recent bottleneck in Ankleshwar chicken. Mukesh *et al.* (2011) studied genetic diversity of Red-Jungle fowl population in India, where they also obtained normal L-shaped mode shift graph showing lack of recent genetic bottleneck as observed in the present study.

Conclusion

The test for genetic equilibrium indicated that most of the markers were not in Hardy-Weinberg Equilibrium, which might be due to limited sample size and selection operating at linked loci as well as sampling from limited area causing relatedness. Moreover, Bottleneck analysis revealed no significant bottleneck in native chicken population within recent past.

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Table 3: Bottleneck analysis for native chicken population of North Gujarat

Models	Sign test	Standardized Difference test	Wilcoxon test
IAM	Hee=15.04	T2=3.614	p (One tail for H deficiency): 0.99993
	Hd=2	p=0.00015	p (One tail for H excess): 0.00008
	He=23		p (Two tails for H excess and deficiency): 0.00016
	p=0.00045		
TPM	Hee=14.87	T2=0.386	p (One tail for H deficiency): 0.83061
	Hd=10	p=0.34992	p (One tail for H excess): 0.17626
	He=15		p (Two tails for H excess and deficiency): 0.35252
	p=0.56437		
SMM	Hee=14.67	T2= -6.759	p (One tail for H deficiency): 0.00937
	Hd=16	p= 0.0000	p (One tail for H excess): 0.99134
	He=09		p (Two tails for H excess and deficiency): 0.01874
	p=0.01857		

(Estimation based on 10000 replications; Hee: Heterozygosity excess expected; He: Heterozygosity excess; Hd: Heterozygosity deficiency; p: Probability; IAM: Infinite Allele Model, TPM: Two Phase Model; SMM: Stepwise Mutation Model, T2: Standard difference test)

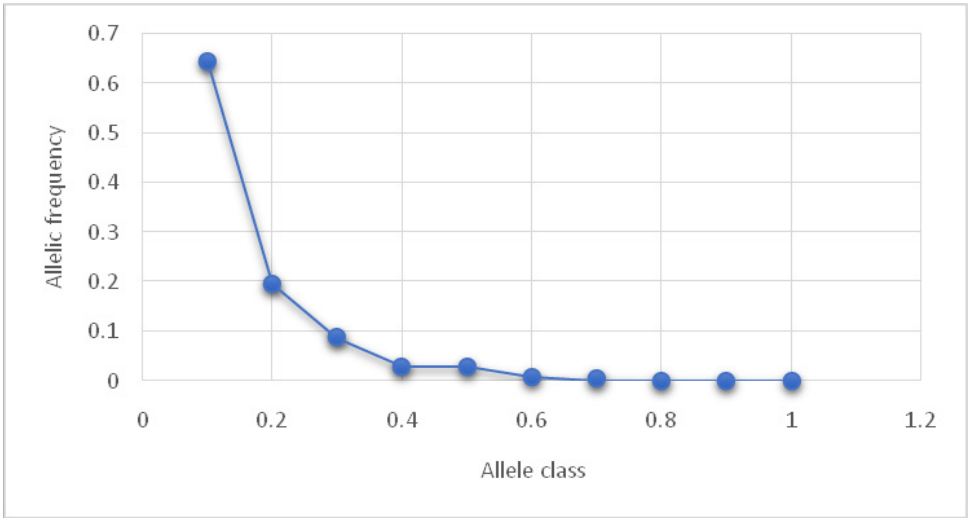

Fig. 1: L-shaped mode shift graph showing lack of recent genetic bottleneck in native chicken population of North Gujarat

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