Population Structure and Genetic Bottleneck Analysis of Ankleshwar Poultry Breed by Microsatellite Markers

A. K. Pandey*, Dinesh Kumar, Rekha Sharma, Uma Sharma, R. K. Vijh and S. P. S. Ahlawat
National Bureau of Animal Genetic Resources, P. O. Box 129, Karnal-132 001, India

ABSTRACT : Genetic variation at 25 microsatellite loci, population structure, and genetic bottleneck hypothesis were examined for Ankleshwar poultry population found in Gujrat, India. The estimates of genetic variability such as effective number of alleles and gene diversities revealed substantial genetic variation frequently displayed by microsatellite markers. The average polymorphism across the studied loci and the expected gene diversity in the population were 6.44 and 0.670±0.144, respectively. The population was observed to be significantly differentiated into different groups, and showed fairly high level of inbreeding (f = 0.240±0.052) and global heterozygote deficit. The bottleneck analysis indicated the absence of genetic bottleneck in the past. The study revealed that the Ankleshwar poultry breed needs appropriate genetic management for its conservation and improvement. The information generated in this study may further be utilized for studying differentiation and relationships among different Indian poultry breeds. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 7 : 915-921)***

Key Words : Ankleshwar, Bottleneck, Genetic Diversity, Microsatellites, Poultry

INTRODUCTION

The ankleshwar poultry breed (*Gallus gallus*) is known to be a breed of Gujarat, India and its native tract is Bharuch (21.41 N latitude and 73.01 E longitude) and Narmada districts (21.38 N latitude and 73.02 E longitude). The name of the breed is derived from the area 'Ankleshwar' of Bharuch district of Gujarat. The breeding area is also extended to Jumbusar, Zagadia, Bharuch, Hansot and Valia of Bharuch and Dediapada, Rajpipla, Tilakwada and Nadod of Narmada district of Gujarat. These poultry birds are being kept mainly by tribes under backyard poultry farming as a livelihood source of income. These birds are maintained without vaccination and medication, and have reasonable feed efficiency as they survive on 25-30 gms of grains, scavenging and maintain excellent fertility (GAU Report, 2003). In spite of its unique characteristics, there is a lack of concern for the conservation and improvement of this breed under field conditions. However, there is worldwide recognition of the need for the conservation of livestock diversity (FAO, 1995) and for characterization of breeds and populations including their genetic differentiation and relationships. These unique characteristics are result of evolutionary forces and their interactions over longer period of time. However, these adaptation and unique characteristics might have been diluted due to intermixing, sub- structuring and/or consequent genetic drift in the population over time. Therefore, an investigation for genetic variation within the breed, and its structure may help to evaluate how likely these factors are operating, and present genetic information to be used in the conservation and improvement of this unique poultry breed.

Of the many genetic markers now available, microsatellite loci are best suited for answering these questions (Goldstein and Pollock, 1997) because of their high variability, high mutation rate, large number, distribution throughout the genome, codominant inheritance and neutrality with respect to selection (Boyce et al., 1996). They are very useful to analyze the degree and pattern of genetic variability within and differences between-populations (Chenyambuga et al., 2004; Li et al., 2004; Wang et al., 2004), genetic differentiation between closely related species (Sun et al., 2004) and parentage verification (Cho and Chol, 2004). The objectives of this study were to study the estimates of genetic variability in the Ankleshwar breed of poultry, its population structure, and to evaluate the genetic bottleneck hypothesis in this breed. The estimated population of this poultry breed is approximately 5,000 (GAU Report, 2003).

MATERIAL AND METHODS

Molecular techniques

Forty-blood samples were collected from genetically unrelated birds from its breeding tract Bharuch and Narmada districts where these birds are confined. Out of 40 blood samples 20 were collected from Ankleshwar, Jumbusar and Zagadia of Bharuch district and rest 20 from Dediapada, Tilakwada and Nadod of Narmada district of Gujarat. Approximately 3 ml blood was collected from each animal's wing vein in vacutainer containing anticoagulant. Genomic DNA was isolated from blood samples as per the

* Corresponding Author: A. K. Pandey. Tel: +91-0184-2267918, Fax: +91-0184-2267654, E-mail: ashwni@yahoo.com
Received October 7, 2004; Accepted February 19, 2005

Table 1. Details of the microsatellite used in the study

S.N.	Loci	Chr.No.	Primer Sequence (5'-3')	Type of repeat	Ta	References
1	MCW41	C3	CCCAATGTGCTTGAATAACTTGGG CCAGATTCTCAATAACAATGGCAG	$(T)_{23}$	55	Crooijmans et al. (1995)
2	MCW48	C4	CGTATAGGAGGGTTTCTGCAGGGA AAGGAGGAACGCACCGCACCTTCT	$(TG)_{17}$	55	Crooijmans et al. (1995)
3	MCW49	C6E1	AGCGGCGTTGAGTGAGAGGAGCGA TCCCCAACCCGCGGAGAGCGCTAT	$(GCA)_n$	55	Crooijmans et al. (1995)
4	MCW59	C1E2	AAGTGCCTTTGCTATCCTGATTGG AACTCCTATTGTGCAGCAGCTTAT	$(T)_{25}$	55	Crooijmans et al. (1995)
5	MCW4	3	GGATTACAGCACCTGAAGCCACTA AAACCAGCCATGGGTGCAGATTGG	$(CA)_{28}$	55	Crooijmans et al. (1995)
6	MCW5	4	ACCTCCTGCTGGCAAATAAATTGC TCACTTTAGCTCCATCAGGATTCA	$(TG)_6AA(TG)_6$ $(A)_{15}(GA)_2(GAA)_7$	55	Crooijmans and Van Kampen (1994)
7	MCW14	6	AAAATATTGGCTCTAGGAACTGTC ACCGGAAATGAAGGTAAGACTAGC	$(CA)_n$	55	Crooijmans and Van Kampen (1994)
8	MCW16	3	ATGGCGCAGAAGGCAAAGCGATAT TGGCTTCTGAAGCAGTTGCTATGG	$(GT)_{15}$	50	Crooijmans and Van Kampen (1994)
9	ADL102	10	TTCCACCTTTCTTTTTTATT GCTCCACTCCCTTCTAACCC	$(GT)_{18}$	47	Cheng and Levin (1995)
10	ADL136	9	TGTCAAGCCCATCGTATCAC CCACCTCCTTCTCCTGTTCA	$(TG)_{10}TC(TG)_{10}$	52	Cheng and Levin (1995)
11	ADL158	10	TGGCATGGTTGAGGAATACA TAGGTGCTGCACTGGAAATC	$(CA)_{12}$	52	Cheng and Levin (1995)
12	ADL171	8	ACAGGATTCTTGAGATTTTT GGTCTTAGCAGTGTTTGTTT	$(TG)_{18}$	46	Cheng and Levin (1995)
13	ADL176	2	TTGTGGATTCTGGTGGTAGC TTCTCCCGTAACACTCGTCA	$(GT)_{12}$	52	Cheng and Levin (1995)
14	ADL210	11	ACAGGAGGATAGTCACACAT GCCAAAAAGATGAATGAGTA	$(CA)_n$	46	Cheng and Levin (1995)
15	MCW7	1	AGCAAAGAAGTGTTCTCTGTTCAT ACCCTGCAAACTGGAAGGGTCTCA	$(TG)_5(TA)_{14}$	60	Wimmer et al. (2000)
16	HUJ7	5	CATAAACTAAAGTCTCAACAC TTCTTCCACCACTCTTGCTA	$(CA)_{10}$	60	Wimmer et al. (2000)
17	HUJ6	3	GGAACATGTAGACAAAAGCA AGCAGTCCATTTCACAGCCA	$(CA)_{15}$	60	Wimmer et al. (2000)
18	HUJ2	17	CATCTCACAGAGCAGCAGTG GAATCCTGGATGTCAAAGCC	$(CA)_{10}$	60	Wimmer et al. (2000)
19	HUJ12	8	GTCTCATGCTATGAGAGTGG CCTCTGGTTGAATCAGTCTG	$(AC)_n$	60	Wimmer et al. (2000)
20	ADL20	1	GCACTCAAAAGAAAACAAAT TAGATAAAAATCCTTCCCTT	$(AC)_{15}$	55	Wimmer et al. (2000)
21	ADL44	12	AAGTGGTTTATTGAAGTAGA CTGTGGTGTTGCGTTAGTTG	$(CA)_n$	60	Wimmer et al. (2000)
22	ADL40	6	TTTCCCCAGATTTACAACTT GCCAGTGATACTCCAGCAGC	$(CA)_n$	49	Wimmer et al. (2000)
23	ADL39	15	GCTACAACGCTTCAAACCTG ACAAACAAACCAAAAAACCT	$(CA)_n$	55	Wimmer et al. (2000)
24	ADL34	E47 W24	AACCTAAAAACTCCTGCTGC GGGAACCTGTGGGCTGAAAG	$(TG)_n$	50	Wimmer et al. (2000)
25	ADL23	5	CTTCTATCCTGGGCTTCTGA CCTGGCTGTGTATGTGTTGC	$(GT)_{12}$		Wimmer et al. (2000)

Chr. No.: Chromosome number; Ta: annealing temperature (°C).

method described by Sambrook et al. (1989) and Pandey et al. (2002). A battery of 25 microsatellite markers (Table 1) was selected based on the guidelines of ISAG & FAO's DADIS programme to generate data in a panel of 40 birds. Polymerase Chain Reaction (PCR) was carried out on about 50-100 ng genomic DNA in a 25 µl reaction volume. The reaction mixture consisted of 200 µM each of dATP, dCTP, dGTP and dTTP, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 1.5 mM $MgCl_2$, 0.75 unit Taq DNA polymerase and 4 ng/µl of each primer (Sigma Genosys) using PTC-200 PCR machine (M J Research). At the end of the reaction, 5.0 µl of stop dye (95% formamide, 0.25% bromophenol blue and 0.25% xylene cyanol) was added and 6 µl of PCR products were loaded on to a 2% agarose gel, electrophoresed and visualized over UV light after ethidium bromide staining to detect the amplification.

The PCR products were resolved on 6% denaturing polyacrylamide gels (Sequi-Gen GT System, Bio-Rad),

ϕX174 *Hinf-I* digest was used as a size standard for sizing PCR products. To visualize the PCR products gels were stained using silver staining (Bassam et al., 2001) and dried between sheets of cellophane paper. The genotypes were scored manually. The size of the alleles was calculated online using 'INCHWORM' programme which estimates the length of the molecule, based on the electrophoretic mobility (http://www.molecularworkshop.com/programs/inchworm.html).

Statistical analysis

For 25 microsatellites loci analyzed, observed and expected heterozygosity estimates were calculated after Levene (1949) and Nei (1973) as implemented in POPGENE software (Yeh et al., 1978). The observed and effective numbers of alleles (Kimura and Crow, 1964) were also calculated using POPGENE software.

The tests for deviation from Hardy-Weinberg equilibrium were derived using the exact tests of POPGENE. Heterogeneity of deviations from Hardy-Weinberg equilibrium among the microsatellite loci was examined by treating the deviations as correlation coefficient and tested accordingly (Barker et al., 2001). As samples were obtained from different localities (two districts), deviations from Hardy-Weinberg equilibrium in the population could be due to genetic differences between subpopulations and a consequent Wahlund effect. Given the observed allele frequencies in each subpopulation, the expected heterozygote deficit due to Wahlund effect can be computed (Li, 1976). Expressing this as a percentage of observed heterozygote deficit then measures the contribution of Wahlund effect to the observed heterozygote deficit. Heterozygote deficiencies were expressed as $D = [H_0-H_E]/H_E$ where H_0 and H_E are the observed and expected frequency of hetrozygotes, respectively.

Tests for pair wise linkage (genotypic) disequilibrium among the microsatellite loci were done using FSTAT version 2.9.3 an update version 1.2 (Goudet, 1995) for 25 microsatellite loci whose genotypes were determined directly. F-statistics were determined after Weir and

Table 2. Measures of genetic variation in Ankleshwar poultry population

SN	Locus	Sample size	Observed number of alleles	Size range (base pairs)	Effective number of alleles	Shannon's information index	PIC	Heterozygosity[a]			Heterozygote deficiency[b]	f-value[c]
								Observed	Expected	Nei's		
1	ADL34	40	9	118-146	5.071	1.844	0.777	0.650	0.813	0.803	-0.2004	0.203
2	ADL39	40	4	152-176	2.617	1.143	0.567	0.700	0.626	0.618	0.1182	-
3	ADL44	40	8	159-195	5.031	1.764	0.773	0.525	0.811	0.801	-0.3526	0.356
4	ADL102	39	8	97-127	3.336	1.489	0.653	0.410	0.709	0.700	-0.4217	0.425
5	ADL210	38	5	115-131	2.085	0.999	0.479	0.342	0.527	0.520	-0.3510	0.354
6	MCW49	40	6	115-130	2.322	1.191	0.537	0.525	0.577	0.569	-0.0901	-
7	ADL20	40	4	95-105	2.714	1.111	0.558	0.850	0.640	0.632	0.3281	-
8	HUJ7	38	5	150-170	2.403	1.118	0.536	0.263	0.592	0.584	-0.5320	0.558*
9	MCW7	39	4	295-317	2.980	1.205	0.604	0.692	0.675	0.666	0.0251	-*
10	MCW48	38	6	187-207	3.856	1.495	0.699	0.763	0.751	0.741	0.0159	-
11	MCW4	40	4	168-200	1.437	0.609	0.283	0.200	0.308	0.304	-0.3506	0.353
12	ADL176	38	5	188-210	3.703	1.436	0.686	0.816	0.740	0.730	0.1027	-
13	MCW59	40	4	156-174	3.313	1.277	0.642	0.450	0.707	0.698	-0.3635	0.366
14	MCW16	40	7	133-155	3.980	1.593	0.717	0.575	0.758	0.749	-0.2414	0.244
15	HUJ6	36	9	101-131	2.536	1.350	0.578	0.528	0.614	0.606	-0.1400	0.142
16	ADL171	37	11	86-122	5.991	2.093	0.829	0.216	0.845	0.833	-0.7443	0.747
17	HUJ12	40	9	112-148	6.387	1.999	0.825	0.675	0.854	0.843	-0.2096	0.212
18	ADL158	38	5	188-198	2.415	1.042	0.505	0.605	0.594	0.586	0.0185	-*
19	ADL136	40	9	132-178	5.405	1.871	0.792	0.475	0.825	0.815	-0.4242	0.428
20	MCW14	39	5	174-188	2.602	1.105	0.541	0.539	0.624	0.616	-0.1362	-
21	ADL40	36	4	195-207	2.123	0.890	0.447	0.389	0.536	0.529	-0.2742	0.278
22	MCW05	34	8	231-291	6.116	1.919	0.816	0.765	0.849	0.837	-0.0989	-
23	ADL23	39	8	164-182	4.844	1.803	0.769	0.333	0.804	0.794	-0.5858	0.589
24	MCW41	40	5	152-172	1.570	0.773	0.346	0.300	0.369	0.365	-0.1869	-
25	HUJ2	38	9	124-142	5.040	1.868	0.780	0.579	0.812	0.802	-0.2869	0.29
26	Mean	38.5	6.44		2.910	1.400	0.623	0.527	0.678	0.670		
27	St. Error		2.162		1.246	0.412		0.187	0.146	0.144		

Effective number of alleles [Kimura and Crow (1964)].

Shannon's Information index [Lewontin (1972)].

[a] Expected heterozygosity were computed using Levene (1949) and Nei's (1973) expected heterozygosity.

[b] Heterozygote deficiencies were expressed as D = (Ho-He)/He, refer the text for symbols used.

[c] f-values (Weir and Cocheran 1984) given for significant tests after Bonferroni corrections.

* Wahlund effects varied from 0.006 to 0.028%; PIC (polymorphic information content).

Table 3. F-statistics analyses for 25 microsatellite loci in Ankleshwar poultry population

Locus*	f (F_{IS})	θ (F_{ST})	F (F_{IT})	Relat	Relatc	R_{st}
ADL34	0.209	0.017	0.195	0.028	-0.486	0.028
ADL39	-0.127	-0.011	-0.114	-0.026	0.205	-0.025
ADL44	0.353	-0.01	0.359	-0.014	-1.12	-0.012
ADL102	0.472	0.168	0.365	0.228	-1.151	-0.020
ADL210	0.357	0.008	0.352	0.011	-1.085	0.015
MCW49	0.115	0.055	0.063	0.099	-0.135	0.050
ADL20	-0.318	0.026	-0.353	0.076	0.522	-0.007
HUJ7	0.56	0.008	0.557	0.01	-2.511	-0.032
MCW7	-0.029	-0.007	-0.022	-0.014	0.044	-0.019
MCW48	0.029	0.092	-0.07	0.18	0.131	-0.023
MCW4	0.429	0.239	0.249	0.334	-0.664	0.040
ADL176	-0.097	0.014	-0.113	0.032	0.203	-0.012
MCW59	0.375	0.028	0.357	0.04	-1.111	-0.024
MCW16	0.248	0.01	0.24	0.016	-0.632	-0.022
HUJ6	0.14	-0.007	0.145	-0.012	-0.34	0.061
ADL171	0.746	-0.002	0.747	-0.002	-5.9	-0.025
HUJ12	0.206	-0.014	0.217	-0.023	-0.556	-0.027
ADL158	0.026	0.092	-0.073	0.179	0.135	0.088
ADL136	0.435	0.025	0.42	0.035	-1.449	-0.017
MCW14	0.133	-0.012	0.143	-0.021	-0.335	-0.005
ADL40	0.302	0.069	0.25	0.107	-0.667	0.196
MCW05	0.112	0.027	0.088	0.048	-0.193	-0.019
ADL23	0.605	0.084	0.569	0.104	-2.643	0.041
MCW41	0.192	0.006	0.187	0.01	-0.461	-0.017
HUJ2	0.315	0.073	0.262	0.11	-0.708	-0.010
Mean	0.240	0.036	0.212	0.058		-0.002
(SE)	0.052	0.010	0.053	0.017		

* Refer to the text for F symbols.

Relat, an estimator of the average relatedness of individuals within samples when compared to whole (Queller and Goodnight's, 1989).

Relatc estmates the inbreeding corrected relatedness (Pamilo, 1985).

[a] Standard errors: estimate from jackknife over loci and significance from t-test using these estimates, $p<0.05$.

R_{st} an estimator of relative genetic differentiation based on fraction of total variance of allele size between two subpopulations.

Cockerham (1984) as used in F-Stat software with Jackknifing procedure applied over loci in deriving significance levels. These parameters of population structure are defined as the correlations between pairs of genes (i) within individuals (F) (ii) between individuals in the same population (θ), and (iii) within individuals within populations (f), and are analogous to Wright's (1978) F_{IT}, F_{ST} and F_{IS} , respectively.

Finally the bottleneck hypothesis was investigated using BOTTLENECK 1.2.01 (Cornuet and Luikart, 1996). The BOTTLENECK tests for the departure from mutation drift equilibrium based on heterozygosity (not hetrozygote), excess or deficiency. This does not require information on historical population sizes or level of genetic variations. It requires only measurement of allele frequencies from 5-20 polymorphic loci in a sample of approximately 20-30 individuals. The bottleneck compares heterozygosity expected (H_E) at Hardy-Weinberg equilibrium to the heterozygosity expected (H_{eq}) at mutation drift equilibrium in same sample, that has the same size and the same number of alleles. All the three models of mutation were used to calculate H_{eq}: the strict one stepwise mutation model (Ohta and Kimura, 1973), the infinite allele model (Kimura and Crow, 1964) and two-phase model (Di Rienzo et al., 1994).

RESULTS AND DISCUSSION

Various measures of genetic variation are presented in the Table 2. The F-statistics estimates are presented in Table 3.

The number of alleles observed across the microsatellite loci studied varied from 4(ADL39, ADL20, MCW7, MCW4, MCW59 and ADL40) to 11 (ADL171) with an overall mean of 6.44±2.162 (Table 2). The observed number of alleles across the loci was more than the effective number of alleles (1.437 to 6.387) as per expectations. The Shannon information index and polymorphic Information Content (PIC) showed that most of the loci were highly informative indicating the high polymorphism across the loci with an overall mean of 1.400 and 0.623, respectively.

Table 4. Mutation-drift equilibrium, heterozygosity excess/deficiency under different mutation models in Ankleshwar poultry population

Methods	Models	Sign test	Standardized	Wilcoxon test
A.	IAM	Hee = 14.84	T_2 = 1.346	P (one tail for H deficiency): 0.96665
Frequency		Hd = 19	p = 0.08911	P (one tail for H excess): 0.03549
method		He = 6		P (two tails for H excess and deficiency): 0.07098
		P = 0.06480		
	TPM	Hee = 14.89	T_2 = -1.287	P (one tail for H deficiency): 0.42660
		Hd = 12	P = 0.9910	P (one tail for H excess): 0.58375
		He = 13		P (two tails for H excess or deficiency): 0.85320
		P = 0.28333		
	SMM	Hee = 14.72	T_2 = -5.510	P (one tail for H deficiency): 0.00279
		Hd = 17	p = 0.00000	P (one tail for H excess): 0.99746
		He = 8		P (two tails for H excess or deficiency): 0.00558
		P = 0.00603		
B.	IAM	Hee = 14.76	T_2 = 0.452	P (one tail for H deficiency): 0.88482
Heterozygosity		Hd = 9	p = 0.32551	P (one tail for H excess): 0.12053
method		He = 16		P (two tails for H excess and deficiency): 0.24107
		P = 0.38685		
	TPM	Hee = 14.79	T_2 = -2.566	P (one tail for H deficiency): 0.07424
		Hd = 16	p = 0.00514	P (one tail for H excess): 0.92954
		He = 9		P (two tails for H excess or deficiency): 0.14848
		p = 0.01648		
	SMM	Hee = 14.80	T_2 = -7.427	P (one tail for H deficiency): 0.00004
		Hd = 19	p = 0.00000	P (one tail for H excess): 0.99996
		He = 6		P (two tails for H excess or deficiency): 0.00009
		p = 0.00038		

Parameters for T.P.M: Variance = 30.00 Proportion of SMM in TPM = 70.00%; Estimation based on 1,000 replications; Hee: Heterozygosity excess expected; Hd: Heterozygosity deficiency; He: Heterozygosity excess; P: Probability; IAM: Infinite allele model, TPM: Two phase model, SMM: Stepwise mutation model.

The average observed heterozygosity was less than the expected (Table 2). The average expected gene diversity (Nei, 1973) within the population ranged from 0.304 (MCW4) to 0.843 (HUJ 12) with an overall mean of 0.670±0.144. Fourteen out of total 25 loci studied showed significant deviations from Hardy Weinberg Equilibrium. All 14 loci showed significant heterozygote deficiency in the Ankleshwar poultry population. Wahlund effects accounted for 0.006 to 0.0281% of the observed heterozygote deficiency at 3 loci.

Significant linkage disequilibrium was detected in the overall microsatellite data for 19 out of 300 loci pairs. The overall means for the F-statistics were significantly different from zero. The relatedness among the individuals in the given sample was also significantly different from zero. The overall Rst, an estimator of genetic differentiation among these samples was -0.002 (Table 3).

Bottleneck analysis as depicted in Table 4 indicates that the observed heterozygosity excess (He) is less than the expected excess heterozygosity (Hee) in 1,000 simulations based on allele frequency and heterozygosity. Here all the 3 models (IAM, TPM and SMM) in both the methods have less He than Hee except one case of IAM in heterozygosity method (He = 16 and Hee = 14.76). The lower magnitude of He with their respective Hee in all the cases (except one) reflects absence of genetic bottleneck in the past population dynamics of Ankleshwar poultry.

When a population experiences a reduction of its effective size, the allele number is reduced faster than the heterozygosity, i.e. the observed heterozygosity is larger than the heterozygosity expected from the observed allele number when the locus is at mutation-drift equilibrium. In addition, qualitative graphical method of Luikart et al. (1998) was used to visualize the allele frequency spectra. The microsatellite alleles were classified in to 10 frequency classes, which allow to check whether the distribution followed the normal L-shaped (Figure 1) form, where alleles with low frequencies (0.01-0.1) are the most abundant. This reflects that the population had not undergone bottleneck at least in the recent past 15-20 generations.

The Ankleshwar poultry breed had substantial genetic variation based on its gene diversity and average number of alleles per locus. The average genetic variation (0.670) observed in this study was in the range of the values reported for other Indian breeds of poultry viz. Kadaknath (0.66), Aseel (0.45), Frizzle-Fowl (0.71), North East (0.56), Nicobari (0.64) and Miri (0.68) (Wimmer et al., 2000; Pandey et al., 2002). The average genetic variation observed in this population was of similar magnitude (0.49-0.77) as reported in African, Asian and South American chicken (Wimmer et al., 2000).

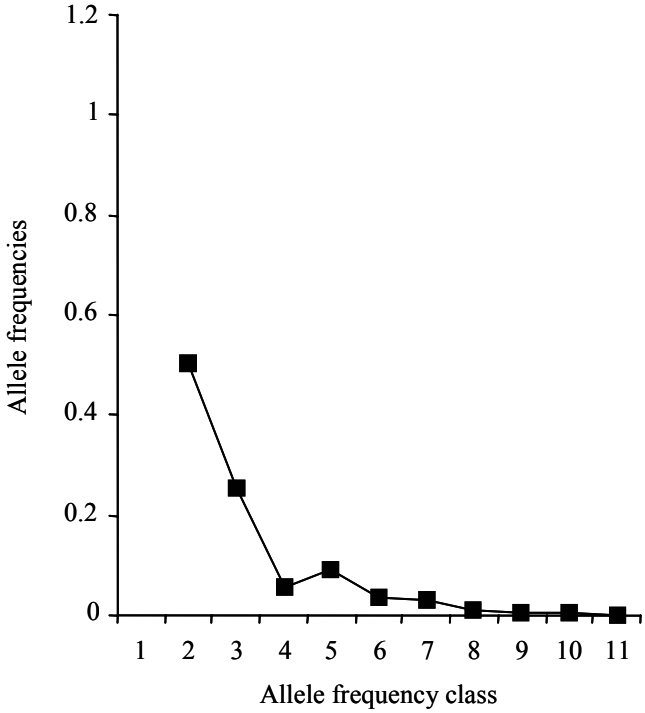

Figure 1. L-shaped mode-shift graph showing lack of bottleneck in the population.

The f (F_{IS}) estimates across 15 out of 25 studied loci were significantly positive (significant heterozygote deficit) based on table wide randomizations ($p<0.05$). The f estimates ranged from 0.142 to 0.589 with an average of 0.240±0.052 (Table 3).

The significant heterozygote deficiency found in ankleshwar population of poultry could be due to one or more of the following reasons: segregation of non-amplifying (null) alleles, Wahlund effects, scoring biases (heterozygotes scored incorrectly as homozygotes) or inbreeding. Distinguishing among these generally is difficult (Christiansen et al., 1974). However, null alleles are most unlikely to be segregating at all the loci. Similarly, scoring bias may be possible for a few loci but not for all loci. Wahlund effects (localities with non-subpopulations) do account for 0.006 to 0.028 of the observed heterozygote deficit for >50% of the loci showing significant heterozygote deficiency. There is strong inbreeding in the population as indicated by high value of f estimate (0.240), presumably resulting from the unplanned and indiscriminate mating prevalent in the breeding tract leading to small effective population size/or mating between relatives and consequent genetic drift. Therefore, inbreeding and Wahlund effects may be most reasonable causes of heterozygote deficit. The non-random association of alleles across the loci was also compatible with genotypic disequilibrium observed in the population. The samples (taken from each of the two districts) were substantially differentiated from each other as indicated by the term theta (θ) and Rst estimates. These estimates are also in accordance with measures of Wahlund effect in the study. F_{IT} estimates revealed significant deviations (heterozygote deficit) from Hardy-Weinberg equilibrium across most of the loci studied. These estimates showed global heterozygote deficit in the population after applying the Bonferroni corrections to different tests.

The results of this study indicated that there is substantial genetic variation and polymorphism across studied loci. The Ankleshwar poultry population was neither in Hardy-Weinberg equilibrium nor in mutation drift equilibrium. The population appears to be divided into significantly differentiated small subpopulations, which resulted in mating among close relatives leading to high level of inbreeding observed in this study. Appropriate breeding strategies should therefore be designed under field conditions for its conservation and improvement of its unique attributes maintained without vaccination and medication, reasonable feed efficiency as they survive on availability of 25-30 gms of grains, scavenging and maintaining excellent fertility 91.34%, (GAU Report, 2003) under the given management and climatic conditions of the region.

REFERENCES

Barker, J. S. F., S. G. Tan, S. S. Moore, T. K. Mukherjee, J. L. Matheson and O. S. Selvaraj. 2001. Genetic variation within and relationships among populations of Asian goats (*Capra hircus*). J. Anim. Breed. Genet. 118:213-233.

Bassam, B. J., G. Coetano-Anolles and P. M. Gresshoff. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal. Biochem. 196:80-83.

Boyce, W. M., P. W. Hedrick, N. E. Muggli-Cockett, S. Kalinowski, M. C. Penedo and R. R. Ramey. 1996. Genetic variation of major histocompatibilty complex and microsatellite loci: a comparison in Bighorn sheep. Genet. 145:421-433.

Cheng, H. H. and I. Levin. 1995. Developing of a genetic map of the chicken with markers of high utility. Poult. Sci. 74:1855-1874.

Chenyambuga, S. W., O. Hanotte, J. Hirbo, P. C. Watts, S. J. Kemp, G. C. Kifaro, P. S. Gwakisa, P. H. Petersen and J. E. O. Rege. 2004. Genetic characterization of indigenous goats of Sub-Saharan Africa using microsatellite DNA markers. Asian-Aust. J. Anim. Sci. 17:445-452.

Cho, G. J. and B. W. Cho1. 2004. Microsatellite DNA typing using 16 markers for parentage verification of the Korean native horse. Asian-Aust. J. Anim. Sci. 17:750-754.

Christiansen, F. B., O. Frydenberg, A. O. Gyldenholm and V. Simonsen. 1974. Genetics of Zoarces populations VI. Further evidence, based on age group samples, of a heterozygote deficit EST III polymorphism. Heriditas. 77:225-236.

Cornuet, J. M. and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genet. 144:2001-2014.

Crooijmans, R. P. M. A. and A. J. A. Van Kampen. 1994. New microsatellite markers on the linkage map of the chicken genome. J. Hered. 85:410-413.

Crooijmans, R. P. M. A., J. J. Van der Poel and M. A. M. Groenen. 1995. Functional genes mapped on the chicken genome. Anim. Genet. 26:73-78.

Di Rienzo, A., A. C. Peterson, J. C. Garza, A. M. Valdes, M. Slatkin, and N. B. Freimer. 1994. Mutational process of simple-sequence repeat loci in human populations. Proc. Natl. Acad. Sci. USA. 91:3166-3170.

FAO. 1995. Global project for the maintenance of domestic animal genetic diversity (MoDAD)-Draft project formulation report, FAO, Rome, Italy.

GAU Report. 2003. Annual Progress Report-2003-04, Network Project on Survey of Poultry Genetic Resources: Ankleshwar poultry, by College of Veterinary Science and Animal Husbandry, Gujarat Agriculture University, Anand-388 110, INDIA.

Goldstein, D. B. and D. D. Pollock. 1997. Launching microsatellites: a review of mutation processes and methods of phylogenetic inference. J. Hered. 88:335-342.

Goudet, J. 1995. FSTAT (version1.2): A computer programme to calculate F-statistics. J. Hered. 86:485-486.

Kimura, M. and J. W. Crow. 1964. The number of alleles that can be maintained in a finite population. Genet. 49:725-738.

Levene, H. 1949. On a matching problem arising in genetics. Ann. Math. Stat. 20:91-94.

Lewontin, R. C. 1972. The apportionment of human diversity. Evol. Biol. 6:381-98.

Li, C. C. 1976. First course in Population Genetics, Boxwood Press, Pacific grove, California.

Li, Changchun, Z. Wang, B. Liu, S. Yang, Z. Zhu, B. Fan, M. Y. Shuhong Zhao and K. Li. 2004. Evaluation of the genetic relationship among ten Chinese indigenous pig breeds with twenty-six microsatellite markers. Asian-Aust. J. Anim. Sci. 17: 441-444.

Luikart, G. and J. M. Cornuet. 1998. Empirical evaluation of a test for identifying recently bottlenecked population form alleles frequency data. Conservation Biology 12:228-237.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA 70:3321-3323.

Ohta, T. and M. Kimura. 1973. A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. Genet. Res. Camb. 22:201-204.

Pamilo, P. 1985. Effect of inbreeding on genetic relatedness. Hereditas. 103:195-200.

Pandey, A. K., M. S. Tantia, D. Kumar, B. Mishra, P. Chaudhary and R. K. Vijh. 2002 Microsatellite analysis of three poultry breeds of India, Asian -Aust. J. Anim. Sci. 15:1536-1542.

Queller, D. C. and K. F. Goodnight. 1989. Estimating relatedness using genetic markers. Evolution 43:258-275.

Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual 2nd ed, Cold spring Harbour, Cold Spring Laboratory Press, NY.

Sun, W., H. Chang, Z. J. Ren, Z. P. Yang, R. Q. Geng, S. X. Lu, L. Du and K. Tsunoda. 2004. Genetic differentiation between sheep and goats based on microsatellite DNA. Asian-Aust. J. Anim. Sci. 17:583-587.

Weir, B. S. and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370.

Wimmer, K., S. Ponsuksili, T. Hardge, A. Valle-Zarate, P. K. Mathur and P. Horst. 2000. Genetic distinctness of African, Asian and South American local chickens. Animal Genetics 31:159-165.

Wang, X., H. H. Cao, S. M. Geng and H. B. Li. 2004. Genetic diversity of 10 indigenous pig breeds in China by using microsatellite markers. Asian-Aust. J. Anim. Sci. 17:1219-1222.

Wright, S. 1978. Evolution and the genetics of populations, Vol. 4. Variability within and among Natural Populations. University of Chicago Press, Chicago.

Yeh, Francis C., R-C. Yang, Timothy B.J. Boyle, Z-H. Ye and Judy X. Mao. 1999. POPGENE version 1.32, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada. (http://www.ualberta.ca/-fyeh/fyeh.).