Microsatellite Analysis of Three Poultry Breeds of India

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ABSTRACT : The genetic variability of three poultry breeds namely Aseel, Miri and Nicobari taken from different geographical locations of India were evaluated using 15 microsatellite loci. No. of alleles varied from 3 to 9 in Aseel, 3 to 8 in Miri and 2 to 7 in Nicobari. Mean PIC values in Aseel, Miri and Nicobari breeds were 0.64, 0.66 and 0.63, respectively. Average unbiased heterozygosity and direct count heterozygosity were 0.65 and 0.59, 0.68 and 0.61, and 0.64 and 0.57 in Aseel, Miri and Nicobari breeds, respectively. High heterozygosity values revealed in this study are indicative of low level of inbreeding, large population size and no or low selection pressure for commercial trait in all three populations. The estimate of genetic distances using Nei's standard, Nei's minimum and Reynold's distance revealed Aseel and Nicobari to be more closely related than Miri breed of poultry. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 11 : 1536-1542)***

Key Words : Poultry, Microsatellite, Genetic Distance

INTRODUCTION

There are 17 defined breeds of domestic fowl (*Gallus domesticus*) in India (Acharya and Bhat, 1984). The three diverse breeds from different geographical location Aseel, Miri and Nicobari were identified for the study. These breeds are of local importance in their regions and are reared as backyard poultry. Several techniques like RAPD, AFLP and Microsatellite analysis are utilized to assess the population relationships. Among these the microsatellite analysis provide even distribution in the genome, and are highly polymorphic and thus are markers of choice for biodiversity analysis. In this study 15 highly polymorphic unlinked microsatellite markers have been utilized for genetic relationship among three native breeds of India.

MATERIALS AND METHODS

Blood samples about 2 ml were collected aseptically from wing vein of three Indian poultry breeds. The samples of Aseel were collected from Bastar district of Chattisgarh and district Khammam of Andhra Pradesh, Miri samples from Dhimaji and North Lakhimpur district of Assam and samples of Nicobari breed were collected from CARI, Portblair (Andaman and Nicobar Islands). Due care was taken that the samples were from unrelated birds to ensure that the samples were random and represent the population. A total of 94 samples were collected, 20 samples of Miri from upper Assam, 38 of Aseel from Bastar region of Chhatisgarh state and 36 of Nicobari from Andaman and Nicobar group of Islands. All the three poultry breeds are predominantly kept by local tribes of the regions. Samples were collected from wing vein using heparin as an anticoagulant. The blood samples were transported to lab at 0-5°C in heparinzed vacutainers. DNA was isolated using standard protocol (Sambrook et al., 1989)

PCR reactions were carried out in a volume of 25 µl containing 50-100 ng genomic DNA 1.5 mM $MgCl_2$, 200 µM of each primer, 200 µM dNTP, one Unit of Taq DNA polymerase. The annealing temperature and Magnesium Chloride concentration were standardized to get the optimal product. Denaturation, annealing and extension steps for 30 cycles were carried out using PTC- 200 PCR machine (MJ Thermal Cycler).

Amplified PCR products were separated on 6% denaturing polyacrylamide gels along with standard DNA markers for sizing. The PAGE was run for sufficiently long period for proper resolution of alleles. The gels were silver stained following standard protocol (Bassam et al., 1991)

The size of the alleles were estimated by making a standard curve taking $\log_{10}$ of the size of standard marker on X-axis and mobility of the DNA on Y-axis. The sizes of the alleles were estimated from the standard curve.

The statistical analysis was carried out using POPGENE software (Yeh et al., 1999). The heterozygosity was calculated using the following formulae given by (Nei, 1978). In order to estimate the genetic variation the observed/direct count and unbiased heterozygosities were calculated for all microsatellite loci in three breeds of poultry.

1. The observed/ direct count heterozygosity was calculated as:

$$1 - \sum_{i=1}^{k-1} X_i^2$$

2. The unbiased heterozygosity

$$H=2n/2n-1 \left[1 - \sum_{i=1}^{k-1} X_i^2\right]$$

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Where:

k=no. of alleles
X_i=frequency of i^{th} allele
X_j=frequency of j^{th} allele

The PIC values (polymorphic information content) was calculated using the formula given by (Botstein et al., 1980)

$$PIC=1-\left(\sum_{i=1}^{k} X_i^2\right) - \sum_{i=1}^{k-1}\sum_{j=i+1}^{k} 2X_i^2 X_j^2$$

The used distance measure was developed by (Nei, 1972) called as Nei's Standard genetic distance Ds was estimated**.** This was calculated as

$$Ds=- \ln I$$

Where I is the identity. Identity is estimated from

$$I=Jxy/\sqrt{Jx\ Jy}$$

Where Jxy and Jx and Jy are the means for all loci of $\Sigma x_i y_i$, Σx_i^2, and Σy_i^2 for each locus. The Nei's minimum genetic distance was calculated (Nei, 1973) as

$$D_m=(J_x+J_y)/2-J_{xy}$$

The effective number of alleles was calculated as given by (Kimura and Crow, 1964). The genetic distances were calculated using Nei's standard (Nei, 1972) and unbiased (Nei, 1978) distance and identity measures. Nei's minimum distances (Nei, 1973; Nei, 1978) were also calculated. The coancestory identity/distance were calculated using Reynold et al., 1983. The phylogenetic tree construction was done using UPGMA algorithm (Swofford and Olsem, 1990). Bootstrapping was used to generate increased confidence in the tree constructed using the original data. The relative lengths of the nodes produced by UPGMA analysis were tested for consistency indices for each node generated by the original data set. The relative strength of the node was tested to find out if the alternative topologies existed (Backeljau et al., 1996). The time of divergence was estimated based on the equation $D=2\ a\ t$, where a is the estimated microsatellite mutation rate and t is time in generations.

RESULTS AND DISCUSSION

The selected microsatellite loci fulfilled conditions like-Mendelian inheritance with PIC value more than 0.6 and were located on different chromosomes/linkage groups (Table 1) and assorted independently (not linked to one another). If these conditions are fulfilled each microsatellite loci represents an independent evolutionary history and is thus suitable for biodiversity analysis. Ten out of 15 loci namely ADL 102, ADL 136, ADL 158, ADL 171, ADL 176, ADL 210, ADL 267, MCW 14, MCW 41, and MCW 59 were from recommended list of loci Domestic Animal Diversity Analysis (Barker et al., 1998), the remaining 5 loci (ADL 20, ADL 23, MCW 5, MCW 7 and MCW 49) were selected after screening published literature.

The number of alleles, effective number of alleles, polymorphic information content (PIC) value, unbiased heterozygosity and direct count heterozygosity in the Aseel, Miri and Nicobari breeds are presented in Table 2, 3 and 4, respectively.

The number of alleles in Aseel breed varied from 3 (MCW 7, ADL 102, ADL 267) to 9 (ADL 136) with average of 4.80. Effective numbers of alleles are less than the observed values averaging 3.09. PIC value varies from 0.49 (MCW 49) to 0.83 (ADL 136) with average of 0.64. Unbiased heterozygosity ranged from 0.49 (MCW 49) to 0.85 (ADL 136) and direct count heterozygosity ranged from 0.25 (ADL 267) to 0.97 (ADL 102) with averages of 0.65 and 0.59, respectively.

The number of alleles in Miri breed ranged from 3 (MCW 7) to 8 (ADL 136,ADL 210) with average of 5.27. Effective numbers of alleles are less than the observed number of alleles with average of 3.39. PIC value varies from 0.35 (MCW 59) to 0.82 (ADL 136) with average of 0.66. Unbiased heterozygosity and direct count heterozygosity ranged from 0.36 (MCW 59) to 0.84 (ADL 136) and 0.29 (MCW 59, ADL 171) to 1.00 (MCW 41) with average of 0.68 and 0.61, respectively.

Nicobari breed revealed average number of alleles as 4.27 with the range from 2 (MCW 14, MCW7, MCW 41, ADL 171) to 7 (ADL 176, ADL 136). In Nicobari the effective number of alleles are less than the observed number of alleles with average of 3.15. PIC value ranged from 0.39 (MCW 14) to 0.82 (ADL 176) with mean of 0.63. Unbiased heterozygosity and direct count heterozygosity ranged from 0.40 (MCW 14) to 0.83 (ADL 176) and 0.23 (MCW 7) to 1.00 (ADL 176) with average of 0.64 and 0.57 respectively.

These results are in conformity with (Wimmers et al., 2000). They also reported high heterozygosity values i.e. 0.66±0.22, 0.45±0.32 and 0.71±0.15 in Indian breeds namely Kadaknath, Aseel and Frizzle Fowl, respectively. The lower heterozygosity value in Aseel in their report may be attributed to the fact that samples in their study were taken from organized flock where these bird may have been subjected to selection pressure and subsequent inbreeding, where as the Aseel poultry in this study were taken from the villages and due care was taken for the samples being

Table 1. Microsatellite markers, primer sequence, location, annealing temperature and $MgCl_2$ concentration

Marker	Primer Sequences	Chromosome No/Linkage Group	Annealing Temperature (°C)	$MgCl_2$ Concentration (mM)
ADL 176	TTGTGGATTCTGGTGGTAGC	E6	52	1.5
	TTCTCCCGTAACACTCGTCA			
ADL 102	TTCCACCTTTCTTTTTTATT	C30	47	1.5
	GCTCCACTCCCTTCTAACCC	E29		
ADL 136	TGTCAAGCCCATCGTATCAC	E5	52	1.5
	CCACCTCCTCCTCCTGTTCA	C10		
ADL 267	AAACCTCGATCAGGAAGCAT	C3	50	2.0
	GTTATTCAAAGCCCCACCAC	E6		
MCW 14	AAAATATTGGCTCTAGGAACTGTC	E11	55	1.5
	ACCGGAAATGAAGGTAAGACTAGC			
ADL 210	ACAGGAGGATAGTCACACAT	E30	46	1.5
	GCCAAAAAGATGAATGAGTA			
MCW 49	AGCGGCGTTGAGTGAGAGGAGCGA	C6	55	1.5
	TCCCCAACCCGCGGAGCGCTAT			
MCW 7	AGCAAAGAAGTGTTCTCTGTTCAT	1	60	0.75
	ACCCTGCAAACTGGAAGGGTCTCA			
MCW 5	ACCTCCTGCTGCAAATAAATTGC	C11	62	1.5
	TCACTTTAGCTCCATCAGGATTCA	E5		
MCW 41	CCCATGTGCTTGAATAACTTGGG	C3	55	1.5
	CCAGATTCTCAATAACAATGGCAG			
ADL 158	TGGCATGGTTGAGGAATACA	C30	52	1.5
	TAGGTGCTGCACTGGAAATC	E29		
ADL 23	CTTCTATCCTGGGCTTCTGA	5	60	1.5
	CCTGGCTGTGTATGTGTTGC			
ADL 20	GCACTCAAAAGAAAACAAAT	1	55	3.0
	TAGATAAAAATCCTTCCCTT			
MCW 59	AAGTGCCTTTGCTATCCTGATTGG	C1	55	1.5
	AACTCCTATTGTGCAGCAGCTTAT	E2		
ADL 171	ACAGGATTCTTGAGATTTTT	E43	46	1.5
	GGTCTTAGCAGTGTTTGTTT			

Table 2. Depicting number of observations, alleles, effective no. of alleles polymorphic information content and heterozygosity values in Aseel breed

S.N.	Locus	No. of Obs.	No. of alleles	Effective No. of alleles	PIC value	Unbiased heterozygosity	Direct count heterozygosity
1.	ADL176	34	8	4.72	0.78	0.79	0.85
2.	ADL102	35	3	2.61	0.61	0.62	0.97
3.	ADL136	35	9	6.22	0.83	0.85	0.42
4.	ADL267	35	3	2.09	0.52	0.52	0.25
5.	MCW14	35	5	2.01	0.50	0.51	0.37
6.	ADL210	35	5	2.44	0.58	0.59	0.60
7.	MCW49	35	4	1.96	0.49	0.49	0.37
8.	MCW7	35	3	2.80	0.64	0.65	0.74
9.	MCW5	35	5	3.74	0.73	0.74	0.77
10.	MCW41	35	4	3.03	0.66	0.67	0.60
11.	ADL158	35	4	2.47	0.59	0.60	0.74
12.	ADL23	31	5	3.13	0.68	0.69	0.48
13.	ADL20	29	4	2.34	0.57	0.58	0.41
14.	MCW59	33	4	3.37	0.70	0.71	0.69
15.	ADL171	35	6	3.36	0.70	0.71	0.57
Average		34.13	4.8	3.09	0.64	0.65	0.59

unrelated but conforming to breed character. However, high inbred lines revealed very high degree of homozygosity with heterozygosity values varying from as low as 0.00 to 0.123 in 23 inbred lines derived from Leghorn, jungle fowl, Fayoumi and Spanish breeds (Zhow and Lamont, 1999).

The use of a mixture of highly variable and less variable microsatellite reduces the risk of overestimating genetic variability, which might occur if only highly variable loci

Table 3. Depicting number of observations, alleles, effective no. of alleles polymorphic information content and heterozygosity values in Miri breed

S.N.	Locus	No. of Obs	No. of alleles	Effective No. of alleles	PIC value	Unbiased heterozygosity	Direct count heterozygosity
1.	ADL176	20	4	2.83	0.64	0.66	0.60
2.	ADL102	20	7	4.88	0.79	0.81	0.75
3.	ADL136	20	8	5.67	0.82	0.84	0.65
4.	ADL267	17	7	4.94	0.79	0.82	0.52
5.	MCW14	16	5	2.71	0.63	0.65	0.43
6.	ADL210	20	8	5.33	0.81	0.83	0.55
7.	MCW49	17	4	2.61	0.61	0.63	0.58
8.	MCW7	20	3	2.57	0.61	0.62	0.95
9.	MCW5	19	5	4.57	0.78	0.80	0.84
10.	MCW41	20	4	2.54	0.60	0.62	1.00
11.	ADL158	19	6	2.45	0.59	0.60	0.52
12.	ADL23	19	5	2.51	0.60	0.61	0.57
13.	ADL20	20	5	3.11	0.67	0.69	0.65
14.	MCW59	17	4	1.54	0.35	0.36	0.29
15.	ADL171	17	4	2.60	0.61	0.63	0.29
Average		18.73	5.27	3.39	0.66	0.68	0.61

Table 4. Depicting number of observations, alleles, effective no. of alleles polymorphic information content and heterozygosity values in Nicobari breed

S.N.	Locus	No. of Obs.	No. of alleles	Effective no of alleles	PIC value	Unbiased heterozygosity	Direct count heterozygosity
1.	ADL176	29	7	5.64	0.82	0.83	1.00
2.	ADL102	31	5	4.22	0.76	0.77	0.64
3.	ADL136	30	7	5.11	0.80	0.81	0.53
4.	ADL267	32	6	3.11	0.67	0.68	0.53
5.	MCW14	29	2	1.66	0.39	0.40	0.41
6.	ADL210	30	6	3.36	0.70	0.71	0.43
7.	MCW49	23	5	4.07	0.75	0.77	0.86
8.	MCW7	30	2	1.99	0.49	0.50	0.23
9.	MCW5	29	4	3.92	0.74	0.75	0.55
10.	MCW41	31	2	1.99	0.49	0.50	0.90
11.	ADL23	29	3	2.49	0.59	0.60	0.55
12.	ADL158	31	5	1.69	0.40	0.41	0.48
13.	ADL20	31	4	3.14	0.68	0.69	0.61
14.	MCW59	29	4	2.92	0.65	0.66	0.41
15.	ADL171	32	2	1.97	0.49	0.50	0.37
Average		29.73	4.27	3.15	0.63	0.64	0.57

are used (Wimmers et al., 2000).

All the three populations revealed higher values for unbiased and direct count heterozygosity. The high heterozygosity values far exceed the values estimated for commercial breeds i.e. 0.40, (Crooijmans et al., 1996). The observed heterozygosity values were less than 0.4 only for 3 loci in Aseel, 2 each in Miri and Nicobari poultry. High heterozygosity value can be attributed to low level of inbreeding, large population size, no or low selection pressure for commercial traits and large number of alleles present in the population. The demographic structure of the three populations reveal that the poultry birds are reared by the tribes as backyard poultry and birds are a part of culture of the tribes and are not subjected to selection of any kind except Aseel where birds are selected for fighting qualities and aggressive behavior.

The effective number of alleles maintained in the population was less than the actual number of alleles. If all the alleles were equally frequent the population of homozygotes would be the reciprocal of number of alleles at this locus maintained in the population. If there is variations in the allele frequency the population of homozygotes will be greater than this. A total of six alleles on 4 loci were specific for Miri poultry while four unique alleles on four loci were found only in Aseel poultry. The data analysis over all the 15 microsatellite loci revealed the Nicobari fowl had no specific alleles and all were shared by Miri and Nicobari poultry. These are not being termed as private alleles because of the small sample size used in the study.

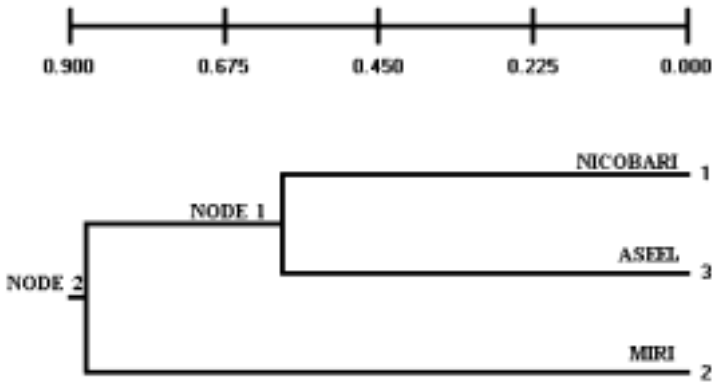

Figure 1. Dendrogram based on standard Nei's distance

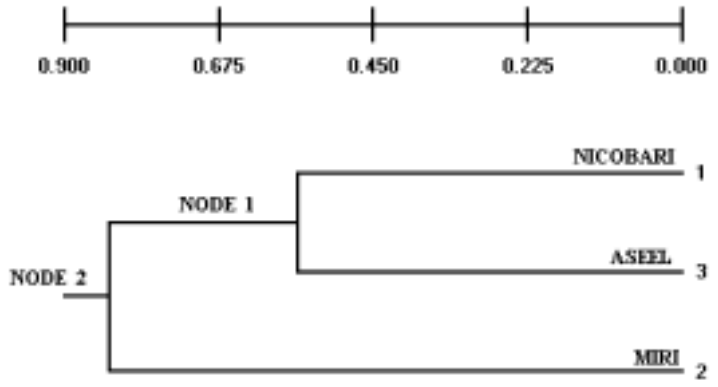

Figure 2. Dendrogram based on unbiased genetic distance

Genetic distance

The calculation of a genetic distance between two populations gives a relative estimate of the time that has passed since the populations existed as single cohesive units. Small estimations of distance may indicate population substructure (i.e., subpopulations in which there is random mating but there is a reduced amount of gene flow). However, small estimation of distance may also be present because the populations are completely isolated but have only been separated for a short period of time. When two populations are genetically isolated, the two processes of mutation and genetic drift lead to differentiation in the allele frequencies until each population is completely fixed for separate alleles. A number of methods have been developed which estimate genetic distance from these allele frequency data like Nei's standard distance, Nei's minimum genetic distance and Reynold's conancestory distance.

The UPGMA cluster analysis using Nei's original distance revealed two nodes. The node one further diverged into populations of Nicobari and Aseel while the population of Miri poultry diverged from node two. To generate a confidence of 95% the data was simulated with 1000 permutations using bootstrapping. None of the bootstraps replicates produced tree containing ties. The consistency index for each node revealed six loci were supporting the node 1 while all the 15 loci supported the node 2. The Nei's distance and identity values and their unbiased estimates are presented in Table 5.

The analysis revealed similar dendrogram for standard and minimum genetic distance. A third analysis of the genetic distance was calculated taking an assumption that if

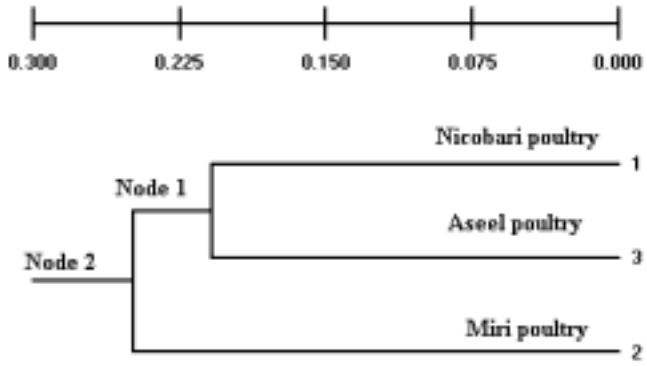

Figure 3. Dendrogram based on genetic distances calculated on basis of coancestory

Table 5. Genetic distance and identity of Nicobari, Miri and Aseel poultry breeds

S.N.	Populations compared	Distance	Identity	Unbiased Distance	Unbiased Identity
1.	Nicobari vs Miri	0.8119	0.4440	0.7685	0.4637
2.	Nicobari vs Aseel	0.5899	0.5544	0.5609	0.5707
3.	Miri vs Aseel	0.9398	0.3907	0.8982	0.4073

the period of differentiation is small then the effect shall be solely due to the coancestery/random genetic drift. The dendrograms produced have also been presented in Figure 1, 2, and 3.

The genetic distances were calculated using allelic frequencies and the dendrograms were constructed. UPGMA clustering using (Nei, 1972) original distance gave two Nodes with distances 0.5899 and 0.8758. The values were 0.5609 and 0.8334 for unbiased distance. The Node 1 had Aseel and Nicobari while Node 2 had all the three populations. The bootstrapping results using 1000 permutations revealed 78.60% similar replicates for Node 1 and 100% for Node 2. No bootstrap replicates produced trees containing ties. The alternative topologies did not exist. Six of the 15 loci (40%) supported Node 1 while all the 15 loci supported Node 2. While 7 of the 15 loci supported node 1 when the procedure of minimum distance was employed. Similar results were also produced when data was analyzed using Reynold's coancestory procedure. The data was further analysed using unbiased values and the genetic distances were obtained for node 1 and node 2. The values obtained were 0.5609 and 0.8334 respectively. Six loci supported the node 1 and all the 15 loci supported the node 2.

All the methods revealed similar phylogenetic tree and has support from the history and geographical location. The closed identity between Nicobari and Aseel supports the view that Aseel poultry birds must have been carried in ships by the traders from the Paradeep seaport (on the east coast), which was an important seaport in earlier times. This is reflected by the fact that these two breeds separated 655 generations ago or approximately 450 years. The geographical location of Miri poultry in Northeastern region of Assam has contiguity with the Aseel breed habitat but separated by more than 1,200 to 1,500 kilometers. The Miri breed might have separated a further 300 generations or 200 years ahead of separation between Aseel and Nicobari breeds.

REFERENCES

Acharya, R. M. and P. N. Bhat. 1984. Livestock and Poultry Genetic Resources in India. IVRI Izatnagar. India.

Bakeljau, T. L., H. D. C. De Bruyn, K. Wolfe, S. Jordaens, Van Dongen and B. Winnepenninckx. 1996. Multiple UPGMA and Neighbour-joining trees and the performance of some computer packages. Mol. Biol. Evol. 13:309-313.

Barker, J. S. F., W. G. Hill, D. Bradley, M. Nei, R. Fries and R. K. Wayne. 1998. Measurement of domestic animal Diversity (MODAD) Original working group report, FAO, Rome.

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.

Botstein, D., R. L. White, M. Skolnick and R. W. Davis. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphism. Am J. Hum. Genet. 32:314-331.

Crooijmans, R. P. M. A., A. B. F. Groen, A. J. A. Van kampen, S. Van Der Beek, J. J. Van Der Poel and M. A. M. Groenen. 1996. Commercial broiler and layer lines estimated using pooled blood samples. Poult. Sci. 75:904-909.

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

Nei, M. 1972. Genetic distance between populations. Am. Naturalist 106(949):283-292.

Nei, M. 1973. The theory of estimation of genetic distance. In: Genetic Structure of Populations, University of Hawaii, Honolulu (Ed. N. Morton). pp. 45-54.

Nei, M. 1978. Estimation of Average heterozygosity and genetic distance from a small number of individuals, Genetics 89: 583-590.

Reynold, J., B. S. Weir and C. C. Cockerham. 1983. Estimation of the coancestory Coefficient basis for a short term genetic distance. Genetics 105:767-779.

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

Swofford, D. L. and G. J. Olsen. 1990. Phylogeny Reconstruction. In: Molecular Systematics (Ed. D. M. Hillis and C. Mortiz). Sinaur Associates Inc. Sunderland.

Wimmers, 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.

Yeh, F. C., T. Boyle, Y. Rongcai, Z. Ye and J. M. Xian. 1999. POPGENE version 3.1

Zhou, H. and S. J. Lamout. 1999. Genetic characterization of biodiversity in highly inbred chicken by microsatellite markers. Animal Genetics 30:256-264.

Figure 1.

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Figure 2.

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