Genetic Relationship in Chicken Breeds Using Molecular Co-ancestry Information

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ABSTRACT : Five chicken populations viz. Chittagong, Ghagus, Kalasthi, Kadaknath, Tellichery were genotyped using 25 highly polymorphic microsatellite loci. White leg horn was taken as an outgroup. To reveal the relationship and distinctiveness among five indigenous breeds various genetic distances based on molecular co-ancestry were estimated and multidimensional scaling was performed. The Ghagus and Kalasthi breeds were closely related and their separation was recent, whereas Chittagong had a remote ancestry with other indigenous chicken populations. (**Key Words :** Chicken, Microsatellites, Coancestry, Genetic Distances)

INTRODUCTION

India has 15 morphologically defined breeds of chicken (Acharya and Bhat, 1984) which contribute 30% to the total egg and meat production of chicken. These indigenous birds are primarily reared as backyard poultry and have their utility in egg, meat and game. These indigenous birds are poor layers producing 50-60 eggs per year (Country report, 2004). The eggs and meat of these indigenous chickens fetch higher prices in the market making them economically viable under low input system. These populations are however important genetic resource owing to their adaptive traits and socio-cultural practices of local communities rearing them. Ghagus and Kalasthi are reared as fighting birds by the tribes. Tellichery and Chittagong are reared for meat and egg. The Kadaknath birds are heavily pigmented in all body parts including abdominal parts known as fibromelanosis. Very little information is available on diversity of chicken breeds of India barring sporadic report on Nicobari Aseel and Miri (Pandey et al., 2002). Kong et al. (2006) and Osman et al. (2006) have studied the genetic variation and relationship in Korean and Japanese chicken breeds, respectively. In this present study we utilized 25 ubiquitously distributed Microsatellites to study the genetic relationship in five breeds of indigenous poultry using genetic distances and molecular co-ancestry information (Alvarez et al., 2005). White leg horn (commercial poultry

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egg laying strain) was taken as an out-group in the study.

MATERIALS AND METHODS

A total of 244 blood samples were collected from 6 populations/breeds from field. The samples were collected from birds conforming to breed characteristics and from a larger area of breeding tract. DNA was extracted from whole blood using the standard protocol (Sambrook et al., 1989). The DNA isolation procedure encompassed lysis of RBC's, digestion of protein using Proteinase K and precipitation of protein using phenol: chloroform: iso-amyl alcohol. DNA was precipitated by gentle addition of 2.5 volumes ethanol and 250 μl of 3 M sodium acetate pH 5.2. The resulting DNA strands were spooled out and washed twice with ice cold 70% ethanol to remove excess salts. DNA was re-dissolved in 500-750 μl of TE buffer pH 8.0. The concentration of DNA was adjudged using comparison with the standard DNA marker concentration on agarose gel. The quality of DNA was checked on 0.8% agarose gel prepared in TAE buffer. The small quantity of blood is required in the case of fowl owing to the fact that the erythrocytes are also nucleated.

A total of 25 primers were taken for the study. These primers were HUJ 002, HUJ 003, LEI 120, LEI 122, LEI 147, LEI 155, LEI 166, LEI 174, LEI 180, LEI 64, LEI 74, LEI 82, LEI 90, LEI 98, MCW 213, MCW 217, MCW 228, MCW 250, MCW 261, MCW 262, MCW 266, MCW 305, MCW 317, MCW 328 and MCW 84. All these primers were present on different chromosomes thus were unlinked
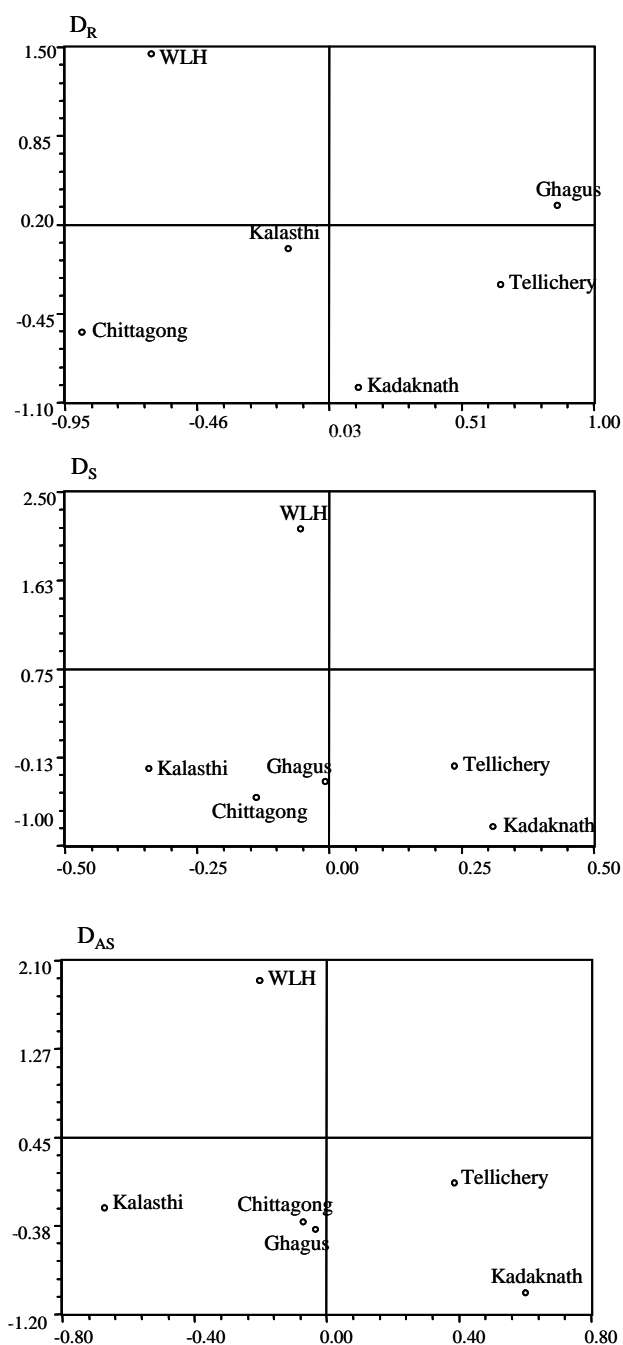
Figure 1. Multidimensional scaling plot constructed using between breeds Reynold's (D_R) Nei's standard (DS) and shared allele (D_{AS}) distances. Dimension 1 is on the x-axis, whereas Dimension 2 is on y-axis.

and covered a large region of chicken genome. The criterion for selection of the microsatellites loci were; 1) in the public domain, 2) mapped and were in linkage disequilibrium, 3) exhibited Mendelian inheritance, 4) at least 4 alleles reported in reference populations. The 5' end of the forward primers was labeled with either FAM or HEX fluorescent dyes.

The PCR conditions were standardized for all of the 25 primer pairs selected for the study. PCR amplification was carried out in 20 μl reaction containing 50 ng genomic DNA, 150 μM dNTP, 4 pmol of forward (labeled) and reverse primers, 1 μ Taq DNA polymerase and 1×reaction buffer (containing 1.5 mM $MgCl_2$). Amplification was carried out in ABI 9700 thermal cycler with initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, annealing temperature for 45 s and 72°C for 45 s. The final cycle was followed by an extension step at 72°C for 10 min. The PCR products were visualized on 2% agarose gel using 1×TAE buffer containing 200 ng/ml of ethidium bromide.

The genotyping was carried out on ABI - 3100 AVANT automated DNA sequencer with ROX 400 HD (Applied Biosystems) as internal lane standard (size standard). The post PCR multiplexing was used to simultaneously genotype 3 or 4 loci depending upon the size and dye label of the PCR product. The sizing and allele calling was performed using Genotyper ver. 3.0 software (Applied Biosystems). The allele data thus generated was used for further statistical analysis.

Statistical analysis

The genetic diversity was assessed by computing heterozygosity (He), heterozygotic deficiency (F_{IS}) and the polymorphic informative content (Botstein et al., 1980). The gene flow among the breeds and genetic differentiation was assessed by computing molecular co-ancestry (f_{ij}, Caballero and Toro, 2002); Kinship distance (Caballero and Toro, 2002); Reynold's distance ($D_R = -\ln\{1-F_{ST}\}$; Reynold et al., 1983), where F_{ST} is the heterozygote deficiency due to population subdivision (Wright, 1969); Nei's standard distance (D_S; Nei, 1987) and shared allele distance (D_{AS}; Chakraborty and Jin, 1993). The within and between breeds molecular co-ancestry and Kinship distance (D_K) was simply computed by averaging the corresponding values for all the within or between population pairs of individuals. Wrights (1969) F-statistics, F_{IS}, F_{ST} and F_{IT} were obtained from the values of mean co-ancestry and inbreeding coefficient. The above computations were carried out using the program MolKin 2.0 (Gutierrez and Goyache, 2005). The multidimensional scaling was carried out using NT sys version 2.0.1.5, which is an exploratory technique for the visualization of proximities in a low dimensional space.

RESULTS

The various parameters describing the variability of the markers for the six breeds of indigenous poultry are presented in Table 1. The 25 microsatellite loci had a total of 358 alleles. The number of alleles ranged from 7 (MCW 84) to 24 (LEI 147). All the microsatellite loci were di-

Table 1. Microsatellite Parameters in different chicken populations

Populations	Ho	He	PIC	k	k'	F_{IS}	D_K'	f_{ii}
Chittagong	0.724	0.804	0.680	10.840	10.389	0.066	0.429	0.196
Ghagus	0.709	0.788	0.653	10.714	10.204	0.080	0.408	0.245
Kadaknath	0.653	0.741	0.590	8.589	8.203	0.127	0.399	0.291
Kalasthi	0.745	0.789	0.647	9.984	9.385	0.047	0.397	0.241
Tellichery	0.661	0.799	0.661	10.199	10.175	0.158	0.444	0.232
WLH	0.728	0.774	0.616	8.448	7.924	0.021	0.379	0.258
Average	0.703	0.810	0.790	14.320	10.749	0.067		

Ho = observed heterozygosity, He = expected heterozygosity, k = mean number of alleles observed, k' = mean number of alleles after rarefaction.
F_{IS} = heterozygote deficiency within population, D_K' = within breed kinship distance, f_{ii} = within population molecular co-ancestry.

Table 2. Pairwise Reynold's distance values (above diagonal) and allele shared distance values (below diagonal) for six poultry populations

Populations	Chittagong	Ghagus	Kadaknath	Kalasthi	Tellichery	WLH
Chittagong		0.023	0.033	0.029	0.025	0.052
Ghagus	0.176		0.032	0.019	0.022	0.061
Kadaknath	0.222	0.197		0.052	0.041	0.074
Kalasthi	0.210	0.130	0.302		0.031	0.064
Tellichery	0.191	0.157	0.237	0.213		0.053
WLH	0.356	0.371	0.397	0.396	0.340	

Table 3. Pairwise Kinship distance values (above diagonal) and molecular co-ancestry values (below diagonal) for six poultry populations

Populations	Chittagong	Ghagus	Kadaknath	Kalasthi	Tellichery	WLH
Chittagong		0.455	0.465	0.459	0.477	0.487
Ghagus	0.183		0.451	0.432	0.461	0.488
Kadaknath	0.192	0.220		0.477	0.484	0.502
Kalasthi	0.172	0.214	0.188		0.470	0.488
Tellichery	0.174	0.204	0.200	0.188		0.497
WLH	0.144	0.157	0.162	0.150	0.160	

nucleotide repeats. The low average number of alleles were found in Kadaknath population (8.59) while the value was 8.45 in the outgroup white leg horn (WLH) population. The values in other populations ranged from 9.98 to 10.84. The average PIC value for the entire data set was quite high (0.790) and all the markers had PIC value higher than 0.640. The marker with lowest values of PIC was MCW84 while LEI147 had PIC of value of 0.898.

The F_{IS} represents the heterozygotic deficiency within the populations while F_{ST} is the measure of population differentiation among the populations and in fact represents that 7.3% of the total variation is attributed to among population and remaining 92.7% within population component. Out group had the least value of F_{IS} of 0.021 being a cross of two commercial lines. The F_{IS} values were lowest for Kalasthi population (0.047) and highest for Tellichery (0.158). The within breed Kinship distance (D_K') was lowest for the outgroup WLH (0.379) while the values were highest for Tellichery population (0.444). The mean molecular co-ancestry and inbreeding values for the whole data set was 0.190 and 0.303 respectively. The outgroup had highest allele sharing genetic distance (D_{AS}) from rest of the five indigenous populations (Table 2). Among the indigenous populations the maximum distance was between

Kalasthi and Kadaknath populations (0.302).

The genetic distances between the populations were estimated for the complete data set. Reynolds' (D_R), Allele sharing (D_{AS}) Kinship (D_K) distance and between breed molecular ancestry, which in fact is a similarity measure, were estimated and have been given in Table 2 and 3. The largest Reynolds distance was found between WLH and all the other indigenous breeds of poultry. Among the indigenous poultry, the maximum distance was between Kalasthi and Kadaknath (0.302) and the lowest between Kalasthi and Ghagus (0.130). The highest distance based on allele sharing was between WLH (outgroup) and all the five other indigenous populations. Among the five indigenous populations the maximum distance was between Kalasthi and Kadaknath (0.052) and least was between Kalasthi and Ghagus. Similar pattern of genetic distances was found in case of Kinship distance; however the maximum Kinship distance was between Kadaknath and Tellichery (0.484), while least was between Kalasthi and Ghagus (0.432).

The molecular co-ancestry, being a measure of similarity, the lowest values were obtained between the outgroup (WLH) and all the five indigenous poultry populations. The maximum value (0.220) was obtained between Ghagus and Kadaknath and least between Kalasthi
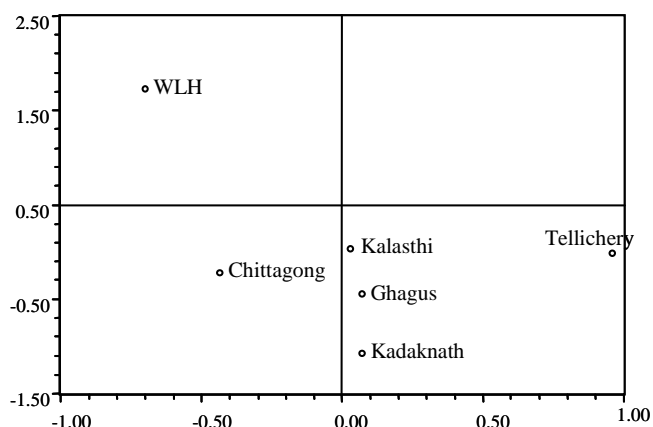
Figure 2. Multidimensional scaling plot constructed using between breeds Kinship distance. Dimension 1 is on the x-axis, whereas Dimension 2 is on y-axis.
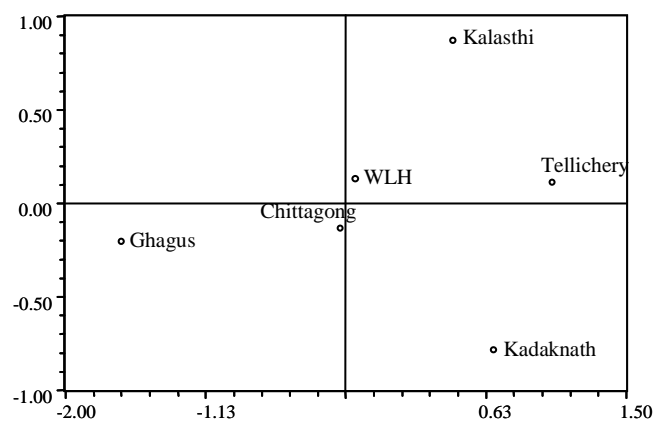

Figure 3. Multidimensional scaling plot constructed using between breeds molecular co-ancestry values. Dimension 1 is on the x-axis, whereas Dimension 2 is on y-axis.

and Chittagong. The molecular co-ancestry value between Kalasthi and Ghagus was 0.214.

DISCUSSION

The PIC value, originally given by Botstein et al. (1980) refers to the values of the marker for detection of polymorphism, depend upon the number of detectable alleles and the distribution of their frequency. It has been proven to be a general measure of information provided by a marker (Guo and Elston, 1999). The molecular ancestry values provide the co-ancestry values which are different from the genetic distance measures and in fact point towards the similarity. The molecular co-ancestry values actually provide the allele frequency in the founder population (Eding and Meuwissen, 2001; Eding et al., 2002), where as the genetic distances including the D_R, D_{AS} and D_S characterize the short term evolution of the populations. The genetic distances including the kinship genetic distance (D_K) are highly dependent on the allele frequencies and are subject to change with the evolutionary process such as genetic drift. However, the information provided by Kinship distance (D_K) the recent between breed differentiation is corrected for allele frequencies before separation of populations (Eding and Meuwissen, 2001).

The various classical genetic distances used in this study provide basically the same information. In all the three genetic distances the WLH, the out group was distinctively placed (Figure 1). The Kalasthi and Chittagong population was closer with Ghagus but distinctively different from Tellichery and Kadaknath. Tantia et al. (2006) reported similar results with chord distance. The plot constructed through the use of Reynolds distance (D_R) gave differentiation of all the five indigenous populations. The D_R has been shown to be an appropriate measure for the short term divergence of the populations. The very low

differentiation among the populations is a result of high with in population variability (Hedrick, 1999; Balloux and Lougon-Moulin, 2002). In case of continuous populations as in the present context there is possibility of some degree of genetic admixture among the breeds. In case of D_R (Figure 1) the WLH is distinctly placed to rest of the populations. Osman et al. (2006) also reported clear separation of native Japanese and foreign chicken breeds. Kalasthi and Ghagus come close to one another followed by Tellichery which can be explained by their geographical proximity. Vijh et al. (2004) also found distinctiveness in four poultry populations with Reynold's distance. Kadaknath and Chittagong are also distinctive from one another and have large geographical distance. The Tellichery and Kadaknath also have higher F_{IS} values (Table 1).

The Kinship distance (D_K) separates the outgroup distinctively to one Quadrant (Figure 2). The Kalasthi and Ghagus population come close to one another and along with Kadaknath and Tellichery are grouped together in a single quadrant. Chittagong is from North East India and occupies a separate distinctive position in the graph. The co-ancestry between the populations remains constant over time after meta-population fragmentation. The genetic distance between populations is determined in terms of co-efficient of Kinship. Its value increases with increase in co-ancestry after separation (Eding and Meuwisson, 2001). The Kinship distance provides an assessment whether the differentiation is remote or recent in origin. Thus D_K plot shows that the differentiation among Kalasthi and Ghagus is recent while that of Kadaknath and Tellichery was remote. The Chittagong has an origin which is remotest among all the five indigenous populations. The plot showing the between population co-ancestry (Figure 3) represents between breed genetic relationship at the moment of separation (ancestral differentiation). The plotted diagram is consistent with the markedly different ancestral genetic

origin for Chittagong poultry.

The present study provides evidence that the Chittagong chicken have separated from rest of the populations since distant time. The populations having geographic proximity (Kalasthi, Ghagus and Tellichery) separated recently. The Kadaknath population revealed high degree of population differentiation owing to preferential selection for fibromelanosis.

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