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Genotypic Comparison of Some Indian Chicken Populations with East Lansing Reference Populations

[1]N. Pirany, [2]K. Kiani and [3]H. Khosravinia
[1]Department of Animal Science, Faculty of Agriculture, University of Tabriz, Iran
[2]Fars Veterinary General Office, Ministry of Agri-Jahad, Shiraz, Iran
[3]Department of Technology of Animal Products, Agriculture Faculty,
Lorestan University, Iran

Abstract: International collaborations to map the chicken genome have been based mostly on 3 reference mapping populations, I in the United States (East Lansing (EL), Michigan) and the other in the United Kingdom (Compton) and III in Wageningen University (WU), the Netherlands. The specific microsatellite markers which are developed by EL and WU are prefixed by ADL and MCW, respectively. To generate the East Lansing mapping family, a single male from the inbred UCD-001 Jungle Fowl (JF) line was mated to a single female from the inbred UCD-003 White Leghorn (WL) line to produce F_1 progeny. For conducting this study, sixteen DNA samples of each six chicken populations with Indian origin, viz, Naked Neck (NN), Giriraja (GR, a synthetic colour strain), randomly chosen local birds (DS), White Silkies (WS) and Commercial Broilers (CB) and layers (CL) were used. Two DNA samples from JF and WL were also obtained from EL and genotyped along Indian chicken populations. Two microsatellite markers from EL (ADL158 and ADL278) and six from WU (MCW5, 16, 29, 37, 104 and MCW119) were used for genotyping of the samples. The JF and WL showed same allele sizes for ADL158 reported by EL (189 bp in JF and 217 bp in WL) but for ADL279, they showed alleles with one and two base pairs less than what was reported by EL for WL (121 bp) and JF (114 bp), respectively. The Indian populations showed alleles of JF for ADL158 with frequency range of 0.0.156-0.625, but for WL varied from 0.0-0.188, respectively. For locus ADL278, Indian populations showed frequency ranges of 0.0-0.313 and 0.0-0.406 for relevant alleles of JF and WL, respectively. White Silkies showed less similarity with WL and in six of the markers, the frequency of alleles were zero. In contrast, CB and NN showed higher similarity with JF than WL. This study revealed that these comparisons could be used to understand evolutionary history of modern chicken populations.

Key words: Chicken, east lansing, india, allele frequency, genotyping

INTRODUCTION

India and the neighboring countries have been referred to as one of the original homes of the Red Jungle Fowl (*Gallus gallus*), the progenitor of domesticated chickens. Over thousands of years of domestication, genetic changes have been accumulated in diverse present-day chicken breeds. Although India has a rich heritage of native poultry germplasm, an estimated 80% of all poultry produced in India is now from foreign breeds (FAO, 1999), which urges decisive measures for conserving native genetic resources. To date, 21 native chicken breeds have been identified in India and a systematic effort should be pursued to characterize and preserve this biodiversity (Sapcota *et al.*, 2002).

Most efforts to map the genomes of birds have concentrated almost exclusively on the domesticated chicken (*Gallus gallus*) and on very few other species. Two reasons for this bias are the importance of chicken as a major source of meat and egg products and as a model of vertebrate development. The first genetic linkage map of the chicken was published in 1936 by Hutt (1936) and represented the first map reported for any domestic farm animal species. Updates of this classical map have been published periodically, with the most recent being that of Bitgood and Somes (1993). The small size of the chicken genome (1.2 billion base-pairs; Bloom *et al.*, 1993) and the ability to isolate DNA from nucleated red blood cells (note: Red blood cells in mammals lack nuclei) make it well suited for gene mapping. Despite these advantages, 6 decades of genetic linkage mapping have produced a limited map. International collaborative efforts to produce a molecular map of the chicken genome have been established in recent years (Burt *et al.*, 1995; 1999).

Corresponding Author: N. Pirany, Department of Animal Science, Faculty of Agriculture, University of Tabriz, Iran

International reference families: International collaborations to map the chicken genome have been based mostly on 2 reference mapping populations, I in the United States (East Lansing, Michigan) and the other in the United Kingdom (Compton). To generate the East Lansing mapping family, a single male from the inbred UCD-001 Jungle Fowl line was mated to a single female from the inbred UCD-003 White Leghorn (WL1) line to produce F1 progeny (Crittenden and others 1993). Two F1 males were individually backcrossed to 10 and 8 UCD-003 WL females to produce 208 and 192 progeny, respectively. Large quantities of blood and DNA from each animal were stored away in aliquots. A subset of 52 progeny (1 F1 male×4 WL females) forms the basic East Lansing mapping panel.

To generate the Compton mapping family, a single line 15I male was mated to a single line N female to produce progeny (Bumstead and Palyga 1992). Unlike the East Lansing mapping family, a single F1 female individual was back-crossed to a line 15I male to generate the mapping family. The consequence of using an F1 female instead of a male in the backcross is that the Z chromosome cannot be mapped in the Compton mapping family; however, the pseudoautosomal region of the W chromosome can be mapped. DNA from a panel of 56 individuals forms the primary mapping panel.

Recently, a third chicken reference family was produced in Wageningen University (WU), The Netherlands, by a collaborative effort between Martien Groenen and Euribrid, a European poultry breeding company. Using 2 commercial broiler lines, 10 F2 families containing a total of 456 progeny were produced (Crooijmans *et al*., 1996). The DNA of this mapping family is not yet publicly available.

To date, the mapped microsatellite markers in EL and WU are widely using in genetic diversity and QTL mapping studies in chicken (Zhou and Lamont, 1999; Tatsuda and Fujinaka, 2001). Because of different evolutionary background and genetic distance between reference populations and modern chicken populations, it is necessary to compare genotypic and allelic profile of those populations. A few studies have been done in this regards. For example, Eugene *et al.* (1999) by using information mapping of EL reference populations assigned additional gene to the linkage groups or chromosomes. Hence, the aim of this study was to compare allelic patterns of some Indian based chicken populations with EL reference populations in order to understand evolutionary history of those populations.

MATERIAL AND METHODS

High genomic DNA was extracted from sixteen (8 Males and 8 Females) bloods of six chicken populations, viz, Naked Neck (NN), Giriraja (GR; a synthetic colour bird), randomly chosen local birds (DS), White Silkies (WS) and Commercial Broilers (CB) and layers (CL). Two DNA samples of White Leghorn (WF) and Red Jungle Fowl (JF) belonging to East Lansing (Michigan) reference populations were also received from USDA-ARS and genotyped along the samples. Population genetic studies were carried out with eight polymorphic microsatellite loci (ADL158, ADl278, MCW5, 16, 29, 37, 104 and 114) which were obtained from Dr. H. Cheng (USDA-ARS) and genotyping was done by ABI377. Different locus and population genetic structure parameters such as observed number of alleles (ONa), effective number of alleles (Ne), number of private alleles (NPa) and observed (Ho) and genetic diversity (G), fixation index (F_{IS}) and Shanon index (H') were calculated according to Nei (1987), Weir and Cockerham (1984) and Sahnon and Weaver (1949), respectively. Genotypic comparison of the Indian chicken populations with EL was only done based on the allelic profile of WL and JF.

RESULTS AND DISCUSSION

In total, 124 alleles were detected at eight microsatellite loci typed in 96+2 individual chickens. The mean number of alleles per locus was 15.5. The MCW37 locus was monomorphic in the WS and CL populations and MCW104 in CL. Number of observed alleles in all samples ranged from three at MCW37 to 27 at MCW5 (Table 1).

The CL population with 4.1 and WS with 4.8 alleles per locus had the lowest mean number of alleles across all the loci (Table 2). The greatest allelic diversity was observed in DS with the mean number of 8.6 alleles per locus. The effective number of alleles showed a similar variation pattern and ranged from 2.7 in CL to 4.7 in DS. Percentage of polymorphic loci was usually 100%, except for CL with 77.8% and WS with 88.9%. The CB population had only four private alleles, whereas DS possessed a maximum of 25 private alleles. Shannon's index also ranged from 0.99 in CL to a maximum of 1.67 in the DS population, with an average of 1.37 over all populations.

Overall, the examined populations showed heterozygosity above 50%. The heterozygosity, averaged over the eight loci for each population, ranged from 0.56 in WS to 0.76 in CB. As expected, the CL population had the lowest gene diversity, with a mean of 0.52 across the loci. Both DS and GR were characterized by the highest gene diversity with a mean of 0.74.

Genotypic comparison of Indian chickens population with JF and WL from EL are presented in Table 3 and 4, respectively. The JF showed 37.5% heterozygosity among the loci. JF showed same size allele for locus ADL158 as EL. Allele frequency for this size ranged from 0.156 (NN) to 0.625 (WS) with an average of 0.453. From Table 1 it is

Table 1: Allelic patterns of microsatellite loci in six Indian chicken populations

Maker name	Allele size range (bp)	Ona*
ADL0158	161-164-176-178-180-182-186-187-189-191-192-193-194-195-196 -197-200-217-221-222	20
ADL0278	108-110-111-112-118-120-122	7
MCW0119	105-107-111-113-115-117-119-121-127-129-131-133-135	13
MCW0005	210-211-212-213-215-216-218-220-221-222-225-231-235-236-237-241-242-244-245 -247-251-252-253-254-255[1]-258-264	27
MCW0016	122-128-129-130-132-134-136-138-140-142-146[2]	11
MCW0029	132-141-142-148-150-152-154-156-158-162-164-166-168-170-172-176-178-184 -188-190-191[3]-193-194-195	25
MCW0037	154-156-158	3
MCW0104	190-196[2]-198-200-202-203-204-206-208-210-212-216-220-222-224-226-228-230	18

* Observed number of alleles. Superscripts are private alleles with frequency over 0.1 in the populations: [1]WS, [2]DS, and [3]CL. The most frequent allele for each locus is shown in bold.

Table 2: Mean (Na) and effective (Ne) number of alleles, heterozygosity (Ho), gene diversity (G), fixation index (F_{IS}), Shannon's index (H'), percentage of polymorphic loci (%P), number of polymorphic loci (Np) and total number of private alleles (Npa) as observed in six chicken populations at eight microsatellite loci

Population	Na	Ne	Ho (SE)	G (SE)	F_{IS}	H'	%P	Np	Npa
NN	5.8	3.7	0.74 (0.051)	0.71 (0.032)	-0.021	1.44	100.00	9	5
WS	4.8	3.4	0.56 (0.086)	0.58 (0.088)	0.069	1.15	88.89	8	8
DS	8.6	4.7	0.63 (0.027)	0.74 (0.035)	0.175	1.67	100.00	9	25
GR	6.4	4.0	0.73 (0.027)	0.74 (0.022)	0.040	1.53	100.00	9	9
CL	4.1	2.7	0.63 (0.128)	0.52 (0.101)	-0.196	0.99	77.78	7	8
CB	6.2	3.6	0.76 (0.070)	0.69 (0.035)	-0.067	1.42	100.00	9	4
Mean	6.0	3.7	0.68 (0.031)	0.66 (0.026)	0.0	1.37	94.44	8.5	9.8

Table 3: Comparative allelic pattern of six Indian chicken populations with JF from EL*

Population	ADL158	ADL278	MCW5	MCW16	MCW29.	MCW37	MCW104	MCW119
JF (bp)	189	112	221-240	136-140	188	154	190	113-117
NN	0 .156	0.031	0.0-0 .0	0 .094-0.031	0 .0	0 .344	0 .250	0 .031-0.438
WS	0 .625	0.281	0.063-0 .0	0.125-0.219	0.0	0.0	0 .469	0 .094-0.406
DS	0.531	0.094	0.156-0 .0	0 .281-0.125	0 .094	0 .25 0	0 .563	0 .031-0.344
GR	0 .250	0.063	0.250 -0.0	0 .313-0.031	0.0	0 .406	0 .094	0.00-0.469
CL	0 .563	0.094	0.469-0.0	0.0-0.0	0 .125	0.0	1.0	0 .031-0.281
CB	0 .594	0.313	0.406-0.0	0 .219-0.250	0 .0	0 .219	0.063	0 .031-0.438

* Allele frequency for relevant allele of JF

Table 4: Comparative allelic pattern of six Indian chicken populations with WL from EL*

Population	ADL158	ADL278	MCW5	MCW16	MCW29	MCW37	MCW104	MCW119
WL (bp)	217	120	242	132	191	154	204-208	113-119
NN	0.0	0.250	0.0	0.125	0.0	0.344	0.0-0.281	0.031-0.281
WS	0.0	0.0	0.031	0.094	0.0	0.0	0.531-0.0	0.094-0.250
DS	0.031	0.344	0.125	0.031	0.0	0.25 0	0.250-0.0	0.031-0.031
GR	0.0	0.406	0.0	0.313	0.0	0.406	0.0-0.188	0.0-0.188
CL	0.188	0.313	0.0	0.594	0.281	0.0	0.0-0.0	0.031-0.0
CB	0.0	0.281	0.0	0.125	0.0	0.219	0.0-0.281	0.031-0.344

* Allele frequency for relevant allele of WL

apparent that this size (189 bp) is the most frequent and common allele to all of the populations. The allele size for ADL278 (112 bp) did not completely meet the allele size of EL (114) and it was 2 bp less than EL was. This allele was common to all of the populations and its frequency was substantial for WS (0.281) and CB (0.313) and for others was less than 0.1. For locus MCW5, JF was heterozygous. It showed 2 alleles of 221 bp, which was the most frequent and 240 bp which was specific to JF only. The frequency of both of the alleles was nearly zero in NN and WS. Again, JF was heterozygous for MCW16 and CB did not showed any of the alleles. The locus MCW29 showed one allele (188 bp) for JF and it was seen in DS and CL only. The JF showed 154 bp allele for MCW0037 but it was absent in WS and CL. The allele frequency for 190 bp of MCW104 except for GR and CB was predominant for others and this for CL was 1.0. The JF was heterozygous for MCW119. The frequency for allele with 113 bp was less than 0.1 but for allele with 117 bp (common allele) was in range of 0.281-0.469.

The WL only showed 25% heterozygosity among the loci. WL showed same size allele (117 bp) for locus ADL0158 as EL. This allele except with low allele frequency for DS (0.031) was specific for CL. The allele size for ADL278 (120 bp) did not completely meet the allele size of EL (121bp) and it was 1 bp less than EL was. Its frequency was zero for WS and substantial for others (0.281-0.406). For locus MCW5, JF was homozygous. It

showed allele with 242 bp, which its frequency in DS was 0.125 and in others except WS (0.031) was zero. Again, JF was homozygous for MCW16 and showed the most frequent allele (132 bp). Its frequency was in range of 0.031 for DS to 0.594 in CL. The locus MCW29 showed one allele (190 bp) for WL and it was only seen in CL with frequency of 0.281. The JF showed 154 bp allele for MCW37 but it was absent in WS and CL. The WL was heterozygous for locus MCW104. It showed two alleles of 204 and 208 bp. The allele with 204 bp was only seen in WS and DD with frequencies of 0.531 and 0.250, respectively and the allele with 208 bp was only seen in NN, GR and CB. The CL did not show any of those alleles. The WL again was heterozygous for MCW119. The allele with 113 bp except GR was seen in other populations and its frequency was less than 0.1. The allele of 119 bp did not see in CL and its frequency in other populations ranged from 0.031-.344.

Eugene *et al.* (1997) with comparative mapping of chicken with East Lansing reference populations assigned 21 additional genes to the linkage groups or chromosomes which five syntenic groups were identified. These groups can be used in future quantitative trait loci investigations.

CONCLUSION

From above results, it can be concluded that from such comparisons it is possible to find out the evolutionary history of modern chicken populations.

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