Estimating microsatellite based genetic diversity in Rhode Island Red chicken

Das, A. K.[1*]; Kumar, S.[1] and Rahim, A.[1, 2]

[1]Molecular Genetics Laboratory, Avian Genetics and Breeding Division, Central Avian Research Institute, Izatnagar 243122, Bareilly, Uttar Pradesh, India; [2]Ph.D. Student in Animal Genetics and Breeding, Division of Animal Genetics, Indian Veterinary Research Institute, Izatnagar 243122, Bareilly, Uttar Pradesh, India

[*]***Correspondence:*** *A. K. Das, Howrah Krishi Vigyan Kendra, Jagatballavpur, Howrah 711408, India. E-mail: dasugenvet@gmail.com*

(Received 2 Dec 2014; revised version 4 May 2015; accepted 26 May 2015)

Summary

This study aimed to estimate microsatellite based genetic diversity in two lines (the selected RIR^S and control line RIR^C) of Rhode Island Red (RIR) chicken. Genomic DNA of 24 randomly selected birds maintained at Central Avian Research Institute (India) and 24 microsatellite markers were used. Microsatellite alleles were determined on 6% urea-PAGE, recorded using GelDoc system and the samples were genotyped. Nei's heterozygosity and Botstein's polymorphic information content (PIC) at each microsatellite locus were estimated. Wright's fixation indices and gene flow were estimated using POPGENE software. All the microsatellite loci were polymorphic and the estimated PIC ranged from 0.3648 (*MCW0059*) to 0.7819 (*ADL0267*) in RIR^S and from 0.2392 (*MCW0059*) to 0.8620 (*ADL0136*) in RIR^C. Most of the loci were highly informative (PIC>0.50) in the both lines, except for five loci in RIR^S and six loci in RIR^C line. Nei's heterozygosity per locus ranged from 0.4800 (*MCW0059*) to 0.8056 (*ADL0267*) in RIR^S and from 0.2778 (*MCW0059*) to 0.875 (*ADL0136*) in RIR^C. Out of 24 loci, 15 (62.5%) in RIR^S and 14 loci (58.33%) in RIR^C revealed moderate to high negative F_{IS} index indicating heterozygote excess for these loci in corresponding lines, but the rest revealed positive F_{IS} indicating heterozygosity deficiency. A mean F_{IS} across the both lines indicated overall 10.77% heterozygosity deficit and a mean F_{IT} indicated 17.19% inbreeding co-efficient favoring homozygosity over the two lines. The mean F_{ST} indicated that 10.18% of the microsatellite variation between the two lines was due to their genetic difference.

Key words: F-statistics, Heterozygosity, Microsatellite allele, Polymorphic information content, Rhode Island Red chicken

Introduction

Microsatellites are of the most popular, numerous, effective and recommended markers because they are highly polymorphic and have repetitive DNA sequences. They are also recommended because they are distributed randomly throughout the genome, display moderate to high levels of variation and co-dominant inheritance (Tautz, 1989). This type of markers have been extensively used in assessing genetic structure, genetic diversity and relationship analyses and are ideal for deciphering genetic variability (Zhou *et al*., 2008). They provide a powerful tool for MAS, QTL research, genome scanning and genetic clustering analysis (Sewalem *et al*., 2002).

One thousand and four hundred fertile eggs of Rhode Island Red (RIR) chicken were imported in 1980 from the USA and hatched at Central Avian Research Institute, Izatnagar, India. The germplasm was subjected to genetic selection. The population was acclimatized and genetically improved over the last 33 years covering 29 generations of selection and being maintained as selected line (RIR^S), and a control line (RIR^C) has been maintained since then. The RIR^S line has shown positive response for egg production after 29 generations of selection based on egg production up to 40 weeks of age, which has, however, been slowing down in the last few generations, probably due to reduction in genetic variability (CARI Annual Report, 2010-11). Faster genetic progress is possible using genomics data, which may impact layer breeding in the future (Albers and Van Sambeek, 2002). Therefore, the present study was carried out to investigate genetic diversity through estimating the Nei's unbiased heterozygosity (Nei's H), polymorphic information content (PIC), Wright's fixation indices and gene flow in the selected and control line of RIR chicken.

Materials and Methods

Twenty-four RIR chicken birds (12 for RIR^S and 12 for RIR^C) maintained at the Experimental Layer Farm of Central Avian Research Institute were randomly chosen. Genomic DNA was extracted using whole blood sample through phenol extraction procedure (Kagami *et al*., 1990), followed by quality checking on 0.7% horizontal submarine agarose gel electrophoresis, purity checking and quantity determination using NanoDrop ND-1000 UV/Vis Spectrophotometer (NanoDrop Technologies, USA). Samples showing intact DNA band and optical density ratio (260 nm: 280 nm) between 1.7 and 1.9 were used in the subsequent experiments. Then, a panel of 24 microsatellite markers, recommended by FAO (2011) and/or used by National Bureau of Animal Genetic Resources, Karnal (India) for genetic characterization, was explored and the chicken specific microsatellite

synthesized primers (Custom Oligos, 0.01 µM) were obtained from M/s Genetics Biotech Asia Pvt. Ltd., New Delhi (India).

PCR reactions were carried out in 25 µL reaction mix prepared by gently mixing 2.5 µL of 10X *Taq* buffer with $MgCl_2$, 2.5 mM of dNTP, 0.8 µM of each primer, 0.75 U *Taq* DNA polymerase and 50 ng template DNA into nuclease free water. The PCR reactions were carried out in a programmable thermal cycler (PTC-200, M. J. Research, USA). The following thermal cycles were used in the PCR reactions: initial denaturing at 94°C for 5 min, followed by 30 cycles of (i) denaturation at 94°C for 1 min, (ii) annealing at optimized annealing temperature for each microsatellite primer pair for 45 s, and (iii) extension at 72°C for 45 s, followed by a final extension at 72°C for 5 min and then 4°C forever.

The molecular sizes of amplified products were determined for their probable sizes through 1.4% horizontal agarose gel electrophoresis by loading approximately 10 µL of PCR product along with 5 µL of 100 bp DNA ladder (Bangalore Genei, India), running it at 2-5 V/cm for 60 min and examining or photographing the products onto gel under UV illumination.

The microsatellite alleles were then identified through running the amplified products on vertical denaturing polyacrylamide gel electrophoresis (PAGE). Approximately 10 µL of the PCR product was mixed with 6X loading dye. The produced mix was denatured at 95°C for 10 min, snapped immediately on ice for 10 min, and was immediately loaded along with 4 µL of 100 bp DNA ladder (Bangalore Genei, India) as molecular size marker on a urea-PAGE gel. Firstly, 30% acrylamide-bisacrylamide (29:1) solution was prepared. Then, urea-PAGE solution was prepared by mixing 10 ml of autoclaved double distilled water, 8.7 ml of 5X TBE buffer and 11.3 ml of 30% acrylamide-bisacrylamide (29:1) solution. In this, 18.0 g of urea was dissolved. Then this urea-PAGE solution was filtered through Whatman paper and kept in refrigerator. The PAGE gel was prepared by adding 250 µL of fresh 10% ammonium per sulphate and 30 µL of tetramethylethylenediamine (TEMED) in 48 ml of chilled transparent urea-PAGE solution. The electrophoresis was carried out at 5-6 V/cm for 4 to 4½ h followed by silver staining (Beidler *et al.*, 1982).

Molecular sizes of various alleles at different microsatellite loci were determined using the Quantity One software on GelDoc 2000 (BioRad, USA). The observed alleles in each sample for each microsatellite loci and its probable genotypes were recorded. Locus specific alleles were identified according to their molecular sizes. Number of observed alleles per each locus, and allelic and probable genotypic frequency were calculated assuming that the population was under Hardy-Weinberg equilibrium. Therefore, the estimated microsatellites' allelic and genotypic frequencies at a locus represented the unbiased maximum likelihood estimates (MLE) of the population examined. The allelic frequency:

$$\tilde{p}_u = \frac{n_u}{2n}$$

with the variance estimated as

$$\text{var}(\tilde{p}_u) = \frac{1}{2n}(p_u + \tilde{p}_{uv} - 2\tilde{p}_u^2)$$

and the genotypic frequency:

$$\tilde{p}_{uv} = \frac{n_{uv}}{n}$$

where,

$\tilde{p}_u$ and $\tilde{p}_{uv}$: The unbiased maximum likelihood estimates of the population frequencies

Average heterozygosity at each locus was calculated according to Nei's (1978) formula:

$$H_i = \frac{2n}{2n-1}\left(1 - \sum_{j=1}^{k} p_j^2\right)$$

where,

p_j: The frequency of the j^{th} allele at i^{th} locus with k number of alleles in the population assumed as under Hardy-Weinberg equilibrium

n: The number of individuals in the population assumed as under Hardy-Weinberg equilibrium

Polymorphic information content at each locus was calculated using the formula described by Botstein *et al*. (1980):

$$PIC_i = 1 - \sum_{i=1}^{k} p_i^2 - \sum_{i=1}^{k-1}\sum_{j=i+1}^{k} 2p_{li}^2 p_{lj}^2$$

where,

P_{li}: The frequencies of i^{th} allele at l^{th} locus with k numbers of alleles in a population

P_{lj}: The frequencies of j^{th} allele at l^{th} locus with k numbers of alleles in a population

The complete genotypic data were subjected to population genetic analysis using POPGENE software (Yeh *et al*., 1999) to estimate Wright's fixation indices and gene flow.

Results

Nei's unbiased heterozygosity index, which is equal to average heterozygosity at a locus, ranged from 0.4800 (*MCW0059*) to 0.8056 (*ADL0267*) in RIR^S line and 0.2778 (*MCW0059*) to 0.875 (*ADL0136*) in RIR^C line with corresponding means ± SE as 0.6753 ± 0.019 and 0.6776 ± 0.028 (Table 1). All the studied loci were polymorphic and their PIC values ranged from 0.3648 (*MCW0059*) to 0.7819 (*ADL0267*) in RIR^S line, with an average of 0.6454 ± 0.023. Corresponding values in the RIR^C line were 0.2392 (*MCW0059*) to 0.8620 (*ADL0136*), with an average of 0.6552 ± 0.031. Most of the loci were highly informative (PIC more than 0.50) in both lines, except for five loci in RIR^S line and six loci in RIR^C line with PIC less than 0.50 (Table 1).

The Wright's fixation indices (F-statistics) and gene flow (Nm) for the examined loci in RIR^S line and RIR^C line are presented in Table 1. F_{IS} statistic estimates variation inside the population i.e. measures the

Table 1: The estimated heterozygosity, polymorphic information contents, F-statistics and gene flow at various microsatellite loci in RIR^S and RIR^C chicken lines

Microsatellite locus	RIR^S line			RIR^C line			RIR^S and RIR^C populations			
	Nei's H	PIC	F_{IS}	Nei's H	PIC	F_{IS}	F_{IS}	F_{IT}	F_{ST}	Nm
MCW0041	0.5417	0.4599	0.3846	0.6111	0.5355	1.0000	0.7108	0.7405	0.1027	2.1842
MCW0043	0.7000	0.6454	-0.1429	0.8056	0.7772	-0.0345	-0.0849	-0.0519	0.0304	7.9706
MCW0044	0.7222	0.6713	-0.3846	0.7778	0.7409	-0.2857	-0.3333	-0.2101	0.0924	2.4545
MCW0048	0.6944	0.6391	-0.4400	0.6975	0.6422	-0.4336	-0.4368	-0.4289	0.0055	45.1000
MCW0049	0.7222	0.6713	1.0000	0.5000	0.4490	1.0000	1.0000	1.0000	0.2414	0.7857
MCW0050	0.6944	0.6391	0.5200	0.5800	0.4918	0.6552	0.5815	0.6332	0.1234	1.7755
MCW0051	0.4861	0.4235	-0.3714	0.5417	0.4599	0.6923	0.1892	0.3878	0.2449	0.7708
MCW0059	0.4800	0.3648	1.0000	0.2778	0.2392	1.0000	1.0000	1.0000	0.4504	0.3050
MCW0071	0.6400	0.5632	1.0000	0.6400	0.5632	1.0000	1.0000	1.0000	0.0303	8.0000
MCW0075	0.7222	0.6713	-0.3846	0.8200	0.7942	-0.2195	-0.2968	-0.2730	0.0184	13.3462
MCW0001	0.8056	0.7772	-0.2414	0.7500	0.7031	-0.3333	-0.2857	-0.1803	0.0820	2.8000
MCW0002	0.7400	0.6918	-0.3514	0.7222	0.6799	-0.3846	-0.3678	-0.3134	0.0398	6.0367
MCW0004	0.8000	0.7716	-0.2500	0.6944	0.6437	-0.4400	-0.3383	-0.1502	0.1406	1.5284
MCW0005	0.7639	0.7260	-0.0909	0.7600	0.7204	-0.0526	-0.0718	-0.0513	0.0191	12.8178
MCW0014	0.5600	0.4992	1.0000	0.5600	0.4992	1.0000	1.0000	1.0000	0.0000	-
MCW0016	0.6389	0.5688	-0.5652	0.6944	0.6391	-0.4400	-0.5000	-0.4472	0.0352	6.8571
ADL0102	0.7200	0.6720	-0.1111	0.7778	0.7409	-0.2857	-0.2018	-0.0700	0.1096	2.0301
ADL0136	0.6806	0.6218	-0.4694	0.8750	0.8620	-0.1429	-0.2857	-0.2203	0.0508	4.6667
ADL0158	0.5972	0.5524	-0.1163	0.8333	0.8119	0.0000	-0.0485	0.0226	0.0679	3.4333
ADL0171	0.6528	0.5786	-0.0213	0.7639	0.7260	-0.3091	-0.1765	-0.1060	0.0599	3.9231
ADL0172	0.7222	0.6713	-0.3846	0.6111	0.5355	-0.6364	-0.5000	-0.3846	0.0769	3.0000
ADL0176	0.5800	0.4918	0.6552	0.4861	0.4235	0.6571	0.6561	0.7595	0.3008	0.5812
ADL0210	0.7361	0.6921	0.7736	0.6771	0.6140	0.0154	0.4103	0.4527	0.0718	3.2302
ADL0267	0.8056	0.7819	0.1724	0.8056	0.7772	-0.2414	-0.0345	0.0164	0.0492	4.8333
Mean ± SE	0.6753 ±0.019	0.6454 ±0.023	0.0909 ±0.1117	0.6776 ±0.028	0.6552 ±0.031	0.1159 ±0.1177	0.1077 ±0.1093	0.1719 ±0.1047	0.1018 ±0.0219	6.0187 ±1.9254

Nei's H, PIC, F_{IS}, F_{IT}, F_{ST} and Nm denote estimates of Nei's unbiased heterozygosity, polymorphic information contents, inbreeding within subpopulation, inbreeding in the total population, inbreeding among subpopulations and gene flow, respectively

deviation from random mating, F_{IT} statistic estimates inbreeding co-efficient of one individual relative to the total populations i.e. measures the deviations from random mating in total and F_{ST} statistic estimates the variation produced by differences between populations i.e. measures the level of genetic divergence among populations. An F_{IS} >0 indicates more inbreeding than expected, which demonstrates deficiency of heterozygotes. F_{IS} <0 indicates less inbreeding than expected, which demonstrates excess of heterozygotes. Out of 24 loci, 15 loci (62.5%) in RIR^S line and 14 loci (58.33%) in RIR^C line revealed moderate to high negative F_{IS} index value indicating heterozygote excess for these loci in corresponding lines. The remaining loci revealed positive F_{IS} estimates indicating heterozygosity deficiency. The F_{IS} value varied from -0.50 (*MCW0016*) to 1.00 (*MCW0059*, *MCW0071* and *MCW0014*). Mean estimated F_{IS} of 0.1077 across both lines indicated overall 10.77% heterozygosity deficit, though the majority of the individual locus demonstrated excess of heterozygotes in a comparatively low percentage. A mean F_{IT} of 0.1719 indicated 17.19% inbreeding co-efficient favoring homozygosity over the two populations. Although 54.14% loci exhibited moderate negative F_{IT} estimates indicative of heterozygosity excess at these loci over the populations. F_{ST} statistic ranged from 0.0 (*MCW0014*) to 0.4504 (*MCW0059*) with a mean value of 0.1018, indicating that 10.18% of microsatellite variation between the two populations was due to their genetic difference. The mean F_{IS}, F_{IT} and F_{ST} for all the markers together in the overall population was 0.1077 ± 0.1093, 0.1719 ± 0.1047 and 0.1018 ± 0.0219, respectively, indicating that the overall population had slight deficit of heterozygotes. An indirect estimation of gene flow (Nm) (Table 1) between two populations was low with an average of 6.0187 ± 1.9254 across the loci excepting loci *MCW0048*, *MCW0075* and *MCW0005*, where it was 45.10, 13.35 and 12.82, respectively.

Discussion

The estimated Nei's H was in accordance with the findings of Hui-Fang *et al*. (2009) in Qingyuan partridge chicken. Polymorphic Information contents (PIC) ranged from moderate to high and more in RIR^C than in RIR^S line, indicating RIR^C as more diverse population. Previously, a range of 0.11 to 0.72 for PIC values was reported in Nigerian and Dahlem Red chickens with corresponding average PIC of 0.72 ± 0.12, 0.6 ± 0.03, 0.36 ± 0.03, 0.18 ± 0.13, 0.47 ± 0.13 and 0.5 ± 0.23 for *MCW0001*, *MCW0004*, *ADL0158*, *ADL0171*, *ADL0210* and *ADL267* loci (Wimmers *et al*., 1999). Parmar *et al*. (2007) reported varied range of PIC values for various microsatellite loci in the Jet Black variety (0.404 to 0.827), Golden variety (0.321 to 0.813) and Penciled variety (0.274 to 0.750) of Kadaknath chicken. Rajkumar *et al*. (2008) reported PIC at 20 markers, out of the 24 microsatellite markers examined in this study, and reported its range from 0.39 (*ADL0158*) to 0.71 (*MCW0005*/*ADL0267*) across different genomes. The average PIC estimate in RIR chicken was 0.66. Babar *et al*. (2012) also studied 9 microsatellite loci including *MCW0041*, *MCW0059*, *MCW0005*, *ADL0102*, *ADL0136*, *ADL0158*, *ADL0171*, *ADL0176* and 2 other loci in 4

varieties of Pakistani Aseel chicken and reported varied mean PIC values (0.67, 0.69, 0.71 and 0.65). The estimated PIC was in the range of the previous reports.

The results of this study were in close agreement with the earlier reports of F_{IS}, F_{IT} and F_{ST} values (Pirany *et al*., 2007; Rajkumar *et al*., 2008; Chatterjee *et al*., 2010) in Indian native and exotic chicken populations. Tadano *et al*. (2007) reported excess of heterozygotes in many cases; however, as a whole, the genetic differences among the chicken lines estimated by the fixation index were high at 29.8%, although higher genetic similarity was observed among White Leghorn lines despite their different breeding histories. Rajkumar *et al*. (2008) used a panel of 20 microsatellite loci the same as in the present study and reported that overall mean F_{IS} ranged from -0.05 (Babcock chicken) to 0.16 (RIR chicken) and the pair-wise estimated F_{ST} ranged from 0.06 (between Aseel and Desi chicken) to 0.14 (between Dahlem Red and Babcock chicken). Wimmers *et al*. (1999) estimated F_{IS} as -0.045 in Dahlem Red chicken and -0.001 in Nigerian RIR at 20 microsatellite loci. Most of the markers examined in the present investigation showed excess of heterozygotes in many cases; however, a small portion (10.18%) of microsatellite variation was due to the genetic differences among the chicken lines used in the study.

Based on the results of this study, it may be concluded that RIR^S line had slight less average heterozygosity than the RIR^C line. Most of the microsatellite loci were moderate to highly informative in both lines. The study suggested that employed set of microsatellite markers may be successfully used in genetic diversity analysis of chicken breeds.

Acknowledgements

The authors wish to sincerely thank the Indian Veterinary Research Institute (IVRI), Izatnagar for providing the IVRI-Senior Research Fellowship to the first author for pursuing his Ph.D. program at this institute. The Director, Central Avian Research Institute (CARI), Izatnagar and Head, Avian Genetics and Breeding Division of this institute are gratefully acknowledged for providing the necessary facilities for this work.

References

Albers, GAA and Van Sambeek, FMJP (2002). Breeding strategies for layers in view of new technologies. In: *Proceedings of 11th European Poultry Conference*. P: 31.

Babar, ME; Nadeem, A; Hussain, T; Wajid, A; Shah, SA; Iqbal, A; Sarfraz, Z and Akram, M (2012). Microsatellite markers based genetic diversity among four varieties of Pakistani Aseel chicken. Pak. Vet. J., 32: 237-241.

Beidler, JL; Hilliard, PR and Rill, RL (1982). Ultrasensitive staining of nucleic acids with silver. Anal. Biochem., 126: 374-380.

Botstein, D; White, RL; Skolnick, M and Davis, RW (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet., 32: 314-331.

CARI Annual Report (2010-11). Central Avian Research Institute (ICAR). Izatnagar, U.P. India. PP: 32-33.

Chatterjee, RN; Sharma, RP; Bhattacharya, TK; Niranjan, M and Reddy, BL (2010). Microsatellite variability and its relationship with growth, egg production, and immuno-competence traits in chickens. Bioch. Genet., 48: 71-82.

FAO (2011). Molecular genetic characterization of animal genetic resources. FAO Animal Production and Health Guidelines. No. 9. Rome.

Hui-Fang, L; Jing-Ting, S; Wei-Tao, S and Kuan-Wei, C (2009). Analysis of genetic diversity in Qingyuan Partridge chickens based on microsatellite markers. J. Anim. Vet. Adv., 8: 454-458.

Kagami, H; Nakamura, H and Tomita, T (1990). Sex identification in chickens by means of the presence of the W chromosome-specific repetitive DNA units. Jap. Poult. Sci., 27: 379-384.

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

Parmar, SNS; Tolenkhomba, TC; Thakur, MS; Joshi, CG; Rank, DN; Solanki, JV; Srivastava, PN and Pillai, PVA (2007). Analysis of genetic relationship among three varieties of indigenous Kadaknath breed using 25 chicken microsatellite markers. Ind. J. Biotech., 6: 205-209.

Pirany, N; Romonov, NM; Ganpule, PS; Devegowda, G and Prasad, TD (2007). Microsatellite analysis of genetic diversity in Indian chicken populations. J. Poult. Sci., 44: 19-28.

Rajkumar, U; Gupta, RB and Reddy, RA (2008). Genomic heterogeneity of chicken populations in India. Asian-Aust. J. Anim. Sci., 21: 1710-1720.

Sewalem, A; Morrice, DM; Law, A; Windsor, D; Haley, CS; Ikeobi, CO; Burt, DW and Hocking, PM (2002). Mapping quantitative trait loci for body weight at three, six and nine weeks of age in a broiler layer cross. Poult. Sci., 81: 1775-1781.

Tadano, R; Nishibori, M; Nagasaka, N and Tsudzuki, M (2007). Assessing genetic diversity and population structure for commercial chicken lines based on forty microsatellite analyses. Poult. Sci., 86: 2301-2308.

Tautz, D (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nuc. Acid. Res., 17: 6463-6471.

Wimmers, K; Ponsuksili, S; Schmoll, F; Hardge, T; Sonaiya, EB; Schellander, K and Horst, P (1999). Application of microsatellite analysis to group chicken according to their genetic similarity. Arch. Anim. Breed., 42: 629-639.

Yeh, FC; Yang, R and Boyle, T (1999). User Manual for POPGENE, Version 1.31, Microsoft Window-Based Freeware for Population Genetic Analysis. A joint Project Development by Francis C. Yeh and Rong-Cai Yang, University of Alberta and Tim Boyle, Centre for International Forestry Research, August 1999.

Zhou, Y; Liu, YP; Kang, L; Meng, XJ; Li, L and Zhu, Q (2008). Correlation between microsatellite loci and onset of lay and egg quality traits in Chinese Silkies, *Gallus gallus*. J. Poult. Sci., 45: 241-248.