

Estimating microsatellite based genetic diversity in Rhode Island Red chicken

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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:

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

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):

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

where,

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

P_j : 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 \pm 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.

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