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Association study between microsatellite genotypes and layer performances in Rhode Island Red chicken*

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ABSTRACT

This investigation aimed to study association between microsatellites and layer performances in Rhode Island Red selected line chicken. Genomic DNA samples isolated from the 12 randomly selected birds were investigated at 24 microsatellite loci. The microsatellite alleles were separated on 6% urea-PAGE and their molecular sizes were estimated. Locus specific alleles were identified according to their sizes, and their association with layer performance traits was assessed by least squares analysis of variance. Analysis revealed that age at sexual maturity of the birds had significant influence of 180bp/190bp and 184bp/196bp microsatellite genotypes in MCW0075 locus. Egg weight at 28th week of age was significantly associated with 210bp/244bp, 216bp/216bp, 216bp/238bp, 222bp/244bp genotypes in MCW0005; and 173bp/173bp, 175bp/175bp, 177bp/177bp in MCW0014. Egg production upto 40 weeks of age was also significantly associated with some genotypes in MCW0044 (133bp/151bp, 136bp/160bp), ADL0102 (136bp/166bp, 146bp/174bp, 166bp/166bp) and ADL0158 (178bp/214bp, 184bp/184bp, 184bp/214bp, 184bp/222bp). MCW0051 (90bp/118bp, 105bp/118bp, 118bp/118bp), MCW0014 (173bp/173bp, 175bp/175bp, 177bp/177bp) and ADL0176 (200bp/200bp, 200bp/236bp, 202bp/202bp) demonstrated significant influences on body weight at 40th week of age. Findings suggested faster genetic progress in RIR flocks by adapting microsatellite genotype based selection.

Key words: Association, Body weights, Egg production, Egg weights, Microsatellite genotypes, RIR chicken

Microsatellite markers are extensively used in assessing genetic structure, genetic diversity and relationship analyses (Zhou *et al.* 2008). They are ideal for deciphering genetic variability (Zhou *et al.* 2008) and provide a powerful tool for marker-assisted selection (MAS) and QTL research (Sewalem *et al.* 2002). The Rhode Island Red (RIR) chicken population brought at the Central Avian Research Institute almost 3 decades ago was well adopted, acclimatized and genetically improved over last 33 years covering 29 generations of selection and being maintained as selected line. The population has shown positive response for egg production on long term selection based on part-period egg production (Anonymous 2011), which however has been slowing down in the last few generations, probably due to reduction in genetic variability (Das *et al.* 2015a). Faster genetic progress is possible using genomics data, which may impact on layer breeding in the future (Albers and Van Sambeek 2002). Hence, the present investigation was carried out with the avowed objective of association study between microsatellites and layer performances in RIR selected line chicken.

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MATERIALS AND METHODS

Birds (12) were randomly chosen from RIR selected line chicken maintained at the Central Avian Research Institute, Izatnagar. Their genomic DNA samples were extracted as detailed in earlier literatures (Das *et al.* 2015a, 2015b) and PCR ready DNA samples were prepared at a concentration of 50 ng/µl. FAO (2011) recommended 24 microsatellite loci as detailed in earlier literatures (Das *et al.* 2015a, 2015b) were used for present study. The chicken specific microsatellite synthesized primers (Custom Oligos, 0.01 µM) were obtained commercially and their annealing temperatures were optimized as per Wimmers *et al.* (2000). The PCR reactions and amplifications were carried out using these DNA samples for each microsatellite marker as detailed in earlier literatures (Das *et al.* 2015a, 2015b). The molecular sizes of amplified products were adjudged for their probable sizes through 1.4% horizontal agarose gel electrophoresis (Das *et al.* 2015a, 2015b). The microsatellite alleles were then identified by running the amplified products on vertical denaturing polyacrylamide gel electrophoresis (6% urea-PAGE) (Das *et al.* 2015a, 2015b) 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. The observed alleles in each sample at each microsatellite loci and its probable genotypes were recorded. Locus specific alleles were identified according to their molecular sizes and assigned names from alphabet A to H in ascending order of their molecular sizes.

The layer performances i.e. body weight at 20th weeks of age (BW20), age at sexual maturity (ASM), egg weights at 28 and 40th week of age (EW28, EW40), body weight at 40th week of age (BW40) and part period egg production upto 40 weeks of age (EP40) were recorded.

The performance data recorded on the experimental birds was analyzed for assessing their association with microsatellite alleles by least squares analysis of variance (Harvey 1990), incorporating microsatellite locus as fixed effect in the statistical model:

$$Y_{jk} = \mu + M_j + e_{jk}$$

where, Y_{jk}, observation of k^{th} individual of j^{th} microsatellite locus; µ, population mean; M_j, fixed effect of j^{th} microsatellite locus; e_{jk}, random error associated with mean zero and variance $ó^2$. Critical Difference (CD) test at the 5% level of probability of significance was performed for assessing critical differences among the least squares means under microsatellite genotypes.

RESULTS AND DISCUSSION

The Least squares analysis of variance elucidates that some specific microsatellite genotypes in loci out of 24 loci investigated in this study had significant ($P<0.05$) influence on performance traits and are presented in Table 1.

Part period egg production upto 40 weeks of age (EP40) was found to be significantly associated with some specific microsatellite genotypes of loci MCW0044, ADL0102 and ADL0158 (Table 1) in agreement to the earlier report (Das *et al.* 2013). Similarly, body weight at 40th week of age (BW40) was significantly influenced by loci MCW0051, MCW0014 and ADL0176; age at sexual maturity (ASM) by MCW0075; and egg weight at 28th week of age (EW28) by MCW0005 and MCW0014 (Table 1) in agreement to the earlier report (Das *et al.* 2013). In accordance to these present findings, previously few workers also reported significant association of some microsatellite alleles/ genotypes with age at sexual maturity (Van Kaam *et al.* 1999, 1998), body weights (Boschiero *et al.* 2009, Jennen *et al.* 2006, Pandya *et al.* 2005, Van Kaam *et al.* 1999, 1998), egg weights (Chatterjee *et al.* 2008, Van Kaam *et al.* 1999, 1998), and egg production (Chatterjee *et al.* 2010, 2008,Wardecka *et al.* 2002, Van Kaam *et al.* 1999, 1998) in different chicken genotypes.

Critical Difference test (Table 2) demonstrated that the microsatellite genotype DG heterozygote of MCW0044 locus had significantly higher EP40 than CF heterozygote of the locus. BD and DD heterozygotes of MCW0051 locus were statistically indifferent for BW40, though they had significantly higher BW40 than CD heterozygote of the locus. CF heterozygote of MCW0075 locus had significantly lower ASM than BE heterozygote of the locus. AE and BD heterozygotes of MCW0005 locus were statistically indifferent for EW28, though they had significantly higher EW28 than either CE heterozygote or BB homozygote of the locus, CE and BB being statistically indifferent. AA homozygote of MCW0014 locus had significantly higher EW28 than either BB or CC homozygotes of the locus, BB and CC being statistically indifferent. Again, AA homozygote of MCW0014 locus had significantly the highest BW40 followed by CC and BB

Table 1. Least squares analysis of variance of various layer performance traits under different microsatellite loci in RIR selected line chicken

Source of variation	df	Mean sum of squares					
		BW20	ASM	EW28	BW40	EW40	EP40
MCW 0044	1	19837.5	600.0	0.04	30104.2	0.04	1410.7**
Remainder	6	28875.0	170.3	7.1	17013.9	14.1	82.6
MCW 0051	2	8583.8	128.4	7.2	50093.8*	8.6	398.4
Remainder	5	35184.0	273.0	5.7	6400.0	13.5	221.8
MCW 0075	1	104.2	864.0*	12.0	8437.5	15.0	600.0
Remainder	6	32163.9	126.3	5.1	20625.0	11.6	217.7
MCW 0005	3	17470.8	170.0	11.9*	25104.2	15.6	292.0
Remainder	4	35168.8	278.0	1.8	14218.8	9.5	257.5
MCW 0014	2	12223.2	71.3	9.1*	36160.7*	10.7	185.1
Remainder	4	38781.3	338.4	1.3	2812.5	13.5	360.8
ADL 0102	2	4580.4	470.5	10.5	13348.2	15.6	595.7*
Remainder	4	41781.3	125.6	2.7	14218.8	9.1	75.5
ADL 0158	3	15640.3	368.1	0.13	21423.6	2.1	563.8*
Remainder	4	36541.7	129.4	10.6	16979.2	19.7	53.6
ADL 0176	2	18223.2	90.8	8.1	33348.2*	10.7	215.7
Remainder	4	35781.3	328.6	1.8	4218.8	13.5	345.5

* $P<0.05$; ** $P<0.01$; *** $P<0.001$. BW20/BW40, body weight in g at 20/40th week of age; ASM, age at sexual maturity in days; EW28/EW40, egg weight in g at 28/40th week of age; EP40, part period egg production in numbers upto 40 weeks of age.

Table 2. The estimated least squares means of various layer performance traits under different microsatellite (MS) genotypes in RIR selected line chicken

Microsatellite loci	MS genotypes Code	allele:allele bp : bp	BW20 (g)	ASM (days)	EW28 (g)	BW40 (g)	EW40 (g) (g)	EP40 (nos.)
MCW 0044	CF	133:151	1600.00±69.37	163.50±5.33	44.83±1.09	1808.33±53.25	53.17±1.54	85.33±3.71[b]
	DG	136:160	1715.00±120.16	143.50±9.23	45.00±1.89	1950.00±92.23	53.00±2.66	116.00±6.43[a]
MCW 0051	BD	90:118	1750.00±187.57	146.00±16.52	48.00±2.39	2050.00±80.00[a]	57.00±3.68	112.00±14.89
	CD	105:118	1616.00±83.89	162.40±7.39	44.00±1.07	1760.00±35.78[b]	52.60±1.65	85.60±6.66
	DD	118:118	1600.00±132.64	155.00±11.68	45.50±1.69	1950.00±56.57[a]	52.50±2.60	102.0±10.53
MCW 0075	BE	180:190	1635.00±126.82	176.50±7.95[b]	47.00±1.60	1900.00±101.55	55.50±2.41	78.00±10.43
	CF	184:196	1626.67±73.22	152.50±4.59[a]	44.17±0.93	1825.00±58.63	52.33±1.39	98.00±6.02
MCW 0005	AE	210:244	1520.00±187.53	169.00±16.67	49.00±1.35[a]	2050.00±119.24	56.00±3.08	84.00±16.05
	BB	216:216	1680.00±187.53	141.00±16.67	42.0±1.35[b]	1850.00±119.24	49.00±3.08	120.0±16.05
	BD	216:238	1750.00±132.61	165.00±11.79	46.50±0.95[a]	1900.00±84.32	56.00±2.18	92.00±11.35
	CE	222:244	1582.50±93.77	157.00±8.34	43.75±0.67[b]	1762.50±59.62	52.00±1.54	89.00±8.02
MCW 0014	AA	173:173	1750.00±196.93	146.00±18.40	48.00±1.15[a]	2050.00±53.03[a]	57.00±3.67	112.0±19.00
	BB	175:175	1565.00±139.25	159.50±13.01	44.50±0.81[b]	1725.00±37.50[c]	52.00±2.60	92.50±13.43
	CC	177:177	1657.50±98.47	158.50±9.20	43.25±0.57[b]	1800.00±26.52[b]	52.00±1.84	90.75±9.50
ADL 0102	AD	136:166	1635.00±144.54	176.50±7.93	47.00±1.16	1900.00±84.32	55.50±2.14	78.00±6.14[b]
	BE	146:174	1582.50±102.20	157.00±5.60	43.75±0.82	1762.50±59.62	52.00±1.51	89.00±4.35[b]
	DD	166:166	1680.00±204.41	141.00±11.21	42.00±1.64	1850.00±119.24	49.00±3.02	120.00±8.69[a]
ADL 0158	CG	178:214	1565.00±135.17	159.50±8.04	44.50±2.31	1725.00±92.14	52.00±3.14	92.50±5.18[b]
	DD	184:184	1715.00±135.17	143.50±8.04	45.00±2.31	1950.00±92.14	53.00±3.14	116.00±5.18[a]
	DG	184:214	1750.00±191.16	184.00±11.38	45.00±3.26	1750.00±130.30	55.00±4.44	72.00±7.32[c]
	DH	184:222	1573.33±110.37	159.33±6.57	45.00±1.88	1883.33±75.23	53.33±2.56	85.00±4.23[bc]
ADL 0176	CC	200:200	1582.50±94.58	157.00±9.06	43.75±0.67	1762.50±32.48[b]	52.00±1.84	89.00±9.29
	CE	200:236	1750.00±189.16	146.00±18.13	48.00±1.35	2050.00±64.95[a]	57.00±3.67	112.0±18.59
	DD	202:202	1715.00±133.76	162.50±12.82	43.50±0.95	1800.00±45.93[b]	52.00±2.60	96.00±13.14

Means within a microsatellite locus having different superscripts differ significantly (P<0.05); BW20/BW40, body weight in g at 20/40th week of age; ASM, age at sexual maturity in days; EW28/EW40, egg weight in g at 28/40th week of age; EP40, part period egg production in numbers upto 40 weeks of age.

homozygotes of the locus. DD homozygote of ADL0102 locus had significantly higher EP40 than either BE or AD heterozygotes of the locus, BE and AD being statistically indifferent. DD homozygote of ADL0158 locus had significantly higher EP40 than either CG or DH or DG heterozygotes of the locus, CG and DH or DH and DG being statistically indifferent. CE heterozygote of ADL0176 locus had significantly higher BW40 than either DD or CC homozygotes of the locus, DD and CC being statistically indifferent. The present findings were in the line of earlier reports of few researchers namely Chatterjee *et al.* (2010, 2008) and Pandya *et al.* (2005). One-to-one correspondence, in the form of significance, between microsatellites and phenotypes like age at sexual maturity, body weights, egg weights and egg production traits may be the informative indicator for elucidating QTL and microsatellite relationships (Chatterjee *et al.* 2010). The genetic principle of significant association of microsatellites and phenotypes is possibly due to the phenomenon of linkage and if the microsatellite be very closely linked (about 20 cM) with a certain phenotype, it will specifically be observed in terms of a significant association (Chatterjee *et al.* 2010) which was observed in the present study, though the linkage analysis (using the CRI-MAP program package) was not carried out in this study because it would thrive to carry out a research with a large number of samples to associate microsatellite alleles with performance traits in more accuracy.

It may be concluded that microsatellite alleles are associated with performance traits suggesting faster genetic progress in layer flocks by adapting microsatellite genotype based selection. The results paved way for utilization of microsatellite markers in molecular breeding for rapid genetic improvement in layer chicken performance. However, further study may be taken on larger sample size to reach definite conclusion.

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