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Impact of microsatellite based selection on grower and layer economic traits in Rhode Island Red chicken

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Growth and egg production traits are quantitative in nature with a continuum between high and low-performing birds, and the regions of the genome that control such traits are termed as quantitative trait loci (QTL). Microsatellites are widely accepted as a marker of choice to detect such QTLs, and the markers linking such QTLs can be used in marker-assisted selection (MAS) program to introduce or preserve beneficial QTL alleles in the population (Debnath *et al.* 2017). They are highly polymorphic, distributed randomly throughout the genome displaying co-dominant inheritance (Tautz 1989) and are extensively used to study genetic structure, variability, diversity and relationship analyses (Das *et al.* 2015). Various microsatellites have been reported to be associated with growth and layer economic traits in chicken and its chromosome number-2 bears several egg-weight/production-associated microsatellites along with other QTLs (Chatterjee *et al.* 2008a). Abasht *et al.* (2006) also reported that this chromosome also harbors genes controlling reproduction.

Rhode Island Red (RIR) is a well-recognized dual-purpose brown-egger chicken breed and useful for backyard poultry production. It has undergone a long-term selection on the basis of 40-weeks part-period egg production over last 33 years covering 30-generations of selection at ICAR-Central Avian Research Institute (CARI, Izatnagar) (Anonymous 2014). For last few generations, part-period egg production has been slowly declining due to reduction in genetic variability, and utilizing genomics data for faster genetic progress was suggested in future (Das *et al.* 2016). The present investigation was carried out to assess the impact of selection based on ADL0176 microsatellite-genotypes and to reveal the underlying association of microsatellite-genotypes at ADL0176 and MCW0044 located on chromosomal number-2 with grower and layer economic traits in RIR chicken.

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A total of 114 birds comprising of 86 females and 28 males from the RIR-selected strain undergoing 30-generations of selection based on 40-weeks part-period egg production were genotyped for ADL0176 microsatellite to produce the experimental birds through molecular breeding. The genotypes were then compared for 40-weeks egg production and the females with DD-genotype followed by CC-genotype revealed the highest production record, whereas females with BB-genotype revealed the lowest record (Debnath *et al.* 2015b). Eleven males belonging to these three homozygous genotypes (DD: 3, CC: 6, BB: 2) were selected and four females having corresponding homozygous genotypes were assigned to each of these males avoiding mating between full and half-sibs with the help of pedigree records, and mated through artificial insemination. The study was conducted on 103 pullets raised out of 286 straight-run chicks (123 under DD-sire family, 148 under CC-sire family, 15 under BB-sire family) (Table 1), obtained in four hatches and subjected to standard housing, feeding and healthcare management and vaccination protocol followed in this institute (Debnath *et al.* 2015a). Following phenol-chloroform extraction method (Kagami *et al.* 1990), genomic DNA was isolated from the blood samples (0.5–1 ml) collected from jugular vein in autoclaved heparinized (5 IU/ml) centrifuge tube. Having assessed the quality of DNA through 0.8% horizontal submarine agarose gel electrophoresis, purity and quantity by NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies Inc., USA) (Debnath *et al.* 2017, Das *et al.* 2015), the samples were subjected to profiling for ADL0176 and MCW0044 microsatellites. The primer sequences: F-5' TTGTGGATTCTGGTGGTAGC3'/ R-5' TTCTCCC-GTAACACTCGTCA3' and F-5' AGTCCGAGCT-CTGCTCGCCTCATA3' / R-5' ACAGTGGCTCAGT-GGGAAGTGACC3', obtained from the published literatures (Chatterjee *et al.* 2010, Chatterjee *et al.* 2008ab) for amplification of corresponding ADL0176 and MCW0044 microsatellite loci were procured from M/s Xcelris Genomics Labs Ltd., Ahmedabad (India). Annealing temperature for each of the primer pairs was optimized (54°C and 63°C, respectively) and PCR amplifications of the DNA samples were carried out for each microsatellite

Table 1. Microsatellite based selection, mating and analyzed genotypes of its progeny-pullets of RIR chicken

Genotype of Sire	Sire No.	No. of Dams per Sire	No. of progeny per Sire family	Pullets raised from straight-run chicks		
				Microsatellite locus	Genotype	Number of pullets
DD	1	4	33	ADL0176	AC	2
	2	4	2		AD	11
	3	4	88		BD	5
CC	4	4	21		CC	41
	5	4	35		CE	2
	6	4	12		DD	31
	7	4	3		EE	9
	8	4	52		Total	101
	9	4	25	MCW0044	AA	37
BB	10	4	15		AB	7
	11	4	0		BB	59
Total no. of straight-run chicks			286		Total	103

Table 2. Estimated P-values of grower and layer economic traits under different microsatellite loci of RIR chicken

Source of variation	df	P values											
		CW	BW8	BW12	BW16	BW20	BW28	BW40	ASM	EW28	EW40	EP28	EP40
Microsatellite ADL0176 locus													
Sire	8	0.0015**	0.042*	0.0011**	0.015*	0.009**	0.038*	0.316 (6)	0.289	0.934	0.550 (6)	0.283	0.251 (6)
Hatch	3	0.227	0.348	0.939	0.779	0.004**	0.813	0.911	0.894	0.428	0.369	0.054	0.405
Genotype	6	0.095	0.420	0.4762	0.714	0.344	0.010** (4)	0.023* (4)	0.453	0.536 (4)	0.004** (4)	0.852 (4)	0.061 (4)
Microsatellite MCW0044 locus													
Sire	8	0.006**	0.065	0.002**	0.019*	0.016*	0.525	0.679 (6)	0.117	0.945	0.960 (6)	0.457	0.393 (6)
Hatch	3	0.355	0.183	0.163	0.415	0.000***	0.174	0.865	0.442	0.840	0.698	0.051	0.325
Genotype	2	0.767	0.825	0.477	0.449	0.231	0.812	0.050*	0.122	0.069	0.269	0.732	0.056

CW, Day-old chick weight; BW, Body weights at different weeks of age; ASM, Age at sexual maturity; EW, Egg weights at different weeks of age; EP, Part-period egg production upto different weeks of age; *P≤0.05, **P≤0.01, ***P≤0.001; Figures within parentheses denote degree of freedom (df): Number of observations varied due to mortality at different ages and sires having single observation were excluded from analysis.

Table 3. Estimated least squares means of grower and layer economic traits under different microsatellite-genotypes of RIR chicken

Microsatellite-genotypes		Least squares means±standard errors											
Code	Allele (bp) : Allele (bp)	CW (g)	BW8 (g)	BW12 (g)	BW16 (g)	BW20 (g)	BW28 (g)	BW40 (g)	ASM (days)	EW28 (g)	EW40 (g)	EP28 (no.)	EP40 (no.)
Microsatellite ADL0176 locus													
AC	225: 202	27.91± 3.08 (2)	449.59± 68.59 (2)	781.79± 121.68 (2)	1144.07± 163.49 (2)	1295.79± 169.00 (2)	1350.45^{c}± 199.02 (2)	1316.04^{b}± 210.30 (2)	131.33± 9.42 (2)	43.65± 3.99 (2)	42.09^{b}± 2.40 (2)	42.23± 10.09 (2)	99.23± 11.72 (2)
AD	225: 196	32.94± 1.42 (11)	509.10± 31.52 (11)	876.95± 55.92 (11)	1225.46± 75.14 (11)	1448.21± 77.67 (11)	1440.37^{c}± 143.98 (3)	1309.50^{b}± 200.02 (2)	140.40± 4.33 (11)	48.43± 2.89 (3)	39.13^{b}± 2.28 (2)	33.09± 7.30 (3)	110.03± 11.15 (2)
BD	210: 196	32.28± 2.23 (7)	490.99± 49.69 (7)	952.47± 88.16 (7)	1251.51± 118.45 (7)	1504.72± 122.44 (7)	–	–	1367.00± 6.82 (7)	–	–	–	–
CC	202: 202	34.53± 0.91 (41)	530.62± 20.14 (41)	923.93± 35.75 (41)	1311.56± 48.03 (41)	1579.93± 49.64 (41)	1743.87^{b}± 90.73 (10)	1884.37^{a}± 220.08 (4)	138.55± 2.77 (41)	43.16± 1.82 (10)	51.73^{a}± 2.51 (4)	34.75± 4.60 (10)	61.32± 12.27 (4)
CE	202: 187	39.27± 3.09 (2)	406.49± 68.76 (2)	838.30± 122.00 (2)	1182.79± 163.91 (2)	1403.96± 169.44 (2)	–	–	153.90± 9.45 (2)	–	–	–	–
DD	196: 196	34.21± 0.94 (31)	528.56± 20.90 (31)	949.66± 37.07 (31)	1327.54± 49.81 (31)	1576.17± 51.49 (31)	1949.69^{a}± 70.68 (18)	1890.97^{a}± 80.84 (16)	135.18± 2.87 (31)	43.76± 1.42 (18)	48.17^{a}± 0.92 (16)	30.97± 3.58 (18)	78.57± 4.51 (16)
EE	187: 187	35.90± 1.80 (9)	543.43± 39.95 (9)	1022.85± 70.88 (9)	1340.19± 95.23 (9)	1598.90± 98.45 (9)	1815.32^{ab}± 147.83 (5)	1969.65^{a}± 160.15 (4)	135.73± 5.49 (9)	43.12± 2.96 (5)	49.91^{a}± 1.82 (4)	32.56± 7.50 (5)	78.67± 8.92 (4)
Microsatellite MCW0044 locus													
AA	144: 144	34.33± 0.96 (37)	527.96± 19.93 (37)	938.98± 35.16 (37)	1318.32± 46.67 (37)	1615.06± 48.70 (37)	1820.40± 97.20 (14)	1505.95^{b}± 192.44 (9)	139.55± 2.69 (37)	41.72± 1.44 (14)	47.16± 2.43 (9)	32.64± 3.84 (14)	82.34± 8.76 (9)
AB	144: 134	33.04± 1.82 (7)	505.70± 37.77 (7)	855.57± 66.65 (7)	1204.87± 88.45 (7)	1464.93± 92.31 (7)	1734.21± 61.32 (2)	1503.34^{b}± 270.94 (2)	142.80± 5.10 (7)	40.02± 4.61 (2)	43.07± 3.42 (2)	30.41± 2.68 (2)	107.65± 12.33 (2)
BB	134: 134	34.31± 0.89 (59)	528.34± 18.38 (59)	930.01± 32.43 (59)	1309.43± 43.04 (59)	1531.28± 44.92 (59)	1791.43± 81.53 (26)	1929.93^{a}± 113.41 (19)	134.40± 2.48 (59)	45.12± 1.21 (26)	49.22± 1.43 (19)	34.29± 3.22 (26)	72.10± 5.16 (19)

CW, Day-old chick weight in gram; BW, Body weights in gram at different weeks of age; ASM, Age at sexual maturity (in days); EW, Egg weights in gram at different weeks of age; EP, Part-period egg production upto different weeks of age; Figures within parentheses denote number of observations. Means with different superscripts within a column under each microsatellite differ significantly ($P \leq 0.05$).

marker as described in earlier literature (Debnath *et al.* 2017). The molecular sizes of the PCR-amplified products were adjudged for their probable sizes through 2% horizontal agarose gel electrophoresis. The microsatellite alleles were then identified by running the PCR-amplified products on horizontal MetaPhore™ agarose gel electrophoresis (3.4% MAGE) (Debnath *et al.* 2019). The molecular sizes of all the alleles at different microsatellite loci were determined using the Quantity One® software 4.6.8 on GelDoc system (Biorad, USA). The observed alleles with its probable genotypes were recorded in each sample at each microsatellite locus (Debnath *et al.* 2019). Locus-specific alleles at each microsatellite locus were identified according to their molecular sizes and noted with alphabet A to E in ascending order of their molecular sizes (Das *et al.* 2015).

Data on grower and layer economic traits were recorded on day-old chick weight (CW, in gram), body weights (BW, in gram) at 16, 28 and 40th weeks of age at empty stomach and egg weight (EW, in gram) at 28 and 40th weeks of age with the help of digital weighing balance. The first-egg laying age was taken as age at sexual maturity (ASM, in days), and the part-period egg production (EP, in numbers) upto 28 and 40-weeks of age were recorded. Data were analyzed by least squares analysis of variance taking sire as random effect, hatch and microsatellite genotype as fixed effect in the linear model using JMP 9.0.0 statistical program package (SAS 2010). The least squares means estimated under the genotypes of each microsatellite locus were compared using critical difference (CD) test at 5% level of probability of significance.

After crossing of the birds selected based on microsatellite ADL0176, 123 chicks were produced under its DD-genotyped sire family, 148 chicks under its CC-genotyped sire family, and 15 chicks under its BB-genotyped sire family (Table 1). Out of 286 straight-run chicks, 103 nos. of female chicks were selected randomly and raised for this work. The pullets raised out of these straight-run chicks when genotyped revealed 7 genotypes at ADL0176 and 3 genotypes at MCW0044 locus and are presented in Table 1. The more resultant genotypes at ADL0176 in the progeny-pullets might be due to the fact that the selected line has undergone 31-generations of selection for egg production which might have resulted in accumulation of some microsatellite alleles over generations, because the number of short-tandem-repeats in a given microsatellite varies greatly among individuals due to gain/ loss of repeat units at a particular locus as a result of slippage during replication or caused by the unequal crossing-over between homologous tandem-repeats.

The results from the least squares analysis of variance of different grower and layer economic traits under different microsatellite genotypes are presented in Table 2 and their estimated means in Table 3. The sire component of variance was found significant on total variance accumulated in CW, BW at 8, 12, 16, 20 and 28th weeks of age of the birds with different ADL0176 microsatellite-genotypes while only in CW, BW at 12, 16 and 20th weeks of age of the birds with different MCW0044 microsatellite-genotypes. Whereas, only housing weight (BW20) had significant hatch component of variance in either case as of the locus concerns. The microsatellite-genotypes at ADL0176 locus demonstrated significant effect on BW28, BW40 and EW40, while the genotypes at MCW0044 had significant effect only on BW40. Previously, microsatellite-genotypes at ADL0176 locus were reported for having significant association with body weights at 40th weeks of age in a selected line of RIR chicken (Das *et al.* 2016) and in three pure lines of White Leghorn populations (Chatterjee *et al.* 2008b). Abasht *et al.* (2006) also reported significant association of microsatellite-genotypes at ADL0176 and MCW0044 with egg numbers and egg weights.

The CD Test clarified that the birds with DD-genotype at ADL0176 locus had the highest BW28 while its AC-genotype had the lowest BW28; although there was no significant difference between DD and EE-genotype, between EE and CC-genotype, and between AD and AC-genotype. The birds with EE-genotype at ADL0176 locus revealed the highest BW40 being indifferent ($P>0.05$) under DD or CC-genotype, while its AD-genotype revealed the lowest BW40 being indifferent ($P>0.05$) under AC-genotype. BB-genotype at MCW0044 locus had the higher BW40 than its AB/AA-genotype. Pullets with CC-genotype at ADL0176 locus laid eggs with the highest weight at 40th weeks of age followed ($P>0.05$) by those with EE and DD-genotype, while the lowest egg weight at 40th weeks of age was found under AD-genotype followed ($P>0.05$) by AC-genotype. Significant association between microsatellite-genotypes and traits like age at sexual maturity, body weights, egg weights and egg production could be quite informative indicator for revealing relationships between QTL and microsatellites (Das *et al.* 2016, Chatterjee *et al.* 2010), probably due to their linkage if the microsatellite be very closely linked (about 20 cM) with the QTL associated to a certain phenotype (Das *et al.* 2016, Chatterjee *et al.* 2010). It may be concluded that significant associations of microsatellite-genotypes with production traits are suggestive of rapid genetic improvement in growth and layer economic traits of RIR chicken by adapting microsatellite-marker based selection strategies.

SUMMARY

The present study aimed to assess impact of selection based on microsatellite-genotypes at ADL0176 and the association of microsatellite-genotypes at ADL0176 and MCW0044 located on chromosome number-2 with growth and layer economic traits in RIR chicken could reveal impact when the sire component influenced the growth and layer economic traits of the progeny-birds with different genotypes at ADL0176 and MCW0044 microsatellites. DD, EE and CC/AD-genotypes at ADL0176 microsatellite had corresponding higher ($P\leq0.05$) BW28, BW40 and EW40 of the progeny than other genotypes, while BB-genotype at MCW0044 had higher ($P\leq0.05$) BW40. Present findings

could suggest the use of microsatellite-marker based selection for faster genetic improvement of economic traits in RIR chicken, provided its validation by taking larger sample sizes.

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