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Association of diversity in microsatellite genotypes with layer traits in Rhode Island Red chicken

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ABSTRACT

Development of vast varieties of high-yielding commercial poultry germplasm can be attributed to rapid selection and controlled breeding. However, their maximum genetic production potential has not been achieved so far. The present study was conducted to analyze polymorphisms in egg production associated microsatellite markers in the sampled population of the selected strain of Rhode Island Red (RIR) chicken and to determine the association between various genotypes of polymorphic markers and layer production traits. One hundred and eleven pullets belonging to five hatches of RIR, maintained at the institute's farm were used and data on body weight at 20 weeks of age (BW20) and layer economic traits. Age at sexual maturity (ASM), egg weight at 28 and 40 weeks of age (EW28, EW40) and egg production up to 40 weeks of age (EP40) were analyzed by least squares analysis of variance taking sire as random and hatch as fixed effects. Average ASM and EP40 were 135.19±1.15 days and 124.55±1.94 eggs, respectively. The BW20 revealed low, but positive genetic as well as phenotypic correlations with EP40 thereby suggested its usefulness as a selection criterion for genetic improvement of egg production. All egg production-associated microsatellite loci revealed polymorphism and exhibited a prevalence of heterozygosity. The studied population demonstrated Hardy-Weinberg disequilibrium. Genotypes at two microsatellite loci ADL0023 and ADL0273 demonstrated significant effects on layer economic traits suggesting the probable usefulness of these microsatellite markers polymorphism in marker-assisted selection for genetic improvement of egg production in RIR chicken.

Keywords: Association, Layer economic traits, Microsatellites, Polymorphisms, Rhode Island Red chicken

Poultry is one of the fastest growing sectors in India which plays a significant role in ensuring food-security. Total poultry population of the country is 851.81 million, which has an increase of 16.81% over previous census of 2012 (20th Livestock census 2019). Annual egg production is 142.77 billion bringing India at 2nd position in egg production (BAHS 2024) with an increase of 3.18% over the previous year. As such per capita availability of eggs is much less than the ICMR recommendation (101 against 180) and faster genetic improvement is very much needed. Rhode Island Red (RIR) chicken breed is a dual-purpose breed and more popular in rural areas being well adapted to local environmental conditions, more disease resistant and preferred by the small flock owners. It has gained more appreciation among consumers due to its brown shelled eggs and better egg producing ability.

Most of the economic traits in layer chicken are related to egg production which is limited to one sex only. The egg production traits are quantitative in nature and regions of the genome that control such traits are termed as quantitative trait loci (QTL). The first genome scans to identify loci affecting egg quality traits have been based on medium-density microsatellite maps (Vikki 2012). Microsatellite (MS) markers are extensively used for determining genetic structure, diversity and relationships (Tautz 1989, Bakare *et al*. 2021). MS markers, by virtue of their co-dominancy and multiple-allelism proved to be efficient in genetic diversity studies, pedigree evaluation and genetic mapping (Ahlawat *et al*. 2004, Debnath *et al*. 2019). Chromosome 1 and 2 bears QTLs related to egg number and egg weight and chromosome 5 carries LOC395381 (ovomucin gene) related to reproductive function in chicken (Abasht *et al*. 2006a, Abasht *et al*. 2006b). Genetic variability at some MS loci have been reported to be associated with egg production traits such as age at sexual maturity, egg weight at 28 and 40 weeks, egg production up to 40 weeks (EP40) of age etc. in RIRs (Das *et al.* 2015, Rahim *et al*. 2023). Hence, the present study was carried out to genotype the egg-production associated microsatellite markers in sampled population of the selected strain of RIR^S chicken and to determine the association between polymorphic markers and layer production traits.

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MATERIALS AND METHODS

Sample collection and genomic DNA isolation: Blood sample collected from experimental RIR^S (n=111) chicken belonging to five hatches. Genomic DNA isolated by Phenol: Chloroform extraction method with slight modification (Sambrook and Russell 2001). Concentration and purity of genomic DNA were assessed by spectrophotometer using NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies Inc., U.S.A.). Quality of extracted genomic DNA was assessed on 0.7% horizontal submarine agarose gel electrophoresis.

PCR amplification: A panel of 10 informative microsatellite markers having known association with egg production traits in various chicken breeds was identified from the published literatures (Chatterjee *et al*. 2008a, Arya 2012, Radwan *et al.* 2014). Forward and reverse primers were synthesized from M/S Xcelris Genomics Labs Ltd., Ahmedabad (India). Nucleotide sequences of primers and corresponding optimized annealing temperature are given in Table 1.

Molecular sizing and genotyping: Molecular sizes of amplified products were adjudged for their probable sizes through 2% horizontal agarose gel electrophoresis. Microsatellite alleles were identified by running amplified products on 3.4% horizontal MetaPhor™ agarose gel electrophoresis (Debnath *et al*. 2017). Molecular sizes (bp) of alleles were determined with help of Quantity One® software 4.6.8 (Bio-Rad Laboratories Inc., U.S.A.) through Gel Doc system. Genotypes of all birds were determined on basis of presence/ absence of microsatellite alleles.

Statistical analysis: Genotypic data were analysed using POPGENE® software 3.1 (Yeh *et al.* 1999). Average heterozygosity and Polymorphic Information Content (PIC) were calculated at each microsatellite locus (Nei 1978, Botstein *et al.* 1980). Performance data were analysed for determining association of various microsatellite genotypes with layer traits by least squares analysis of variance (LS ANOVA) incorporating microsatellite genotype as fixed effect in the model. The microsatellite-wise analysis was done for all the ten microsatellites using following statistical model:

$$Y_{ijkl} = \mu + S_i + H_j + M_k + e_{ijkl}$$

Where,

Y_{ijkl} : value of growth and layer economic traits measured on ijklth individual,

μ : Overall mean,

S_i : Random effect of ith sire,

H_j : fixed effect of jth hatch (j=1 - 5),

M_k : effect of kth genotype of a particular microsatellite marker (k=1 - no. of alleles),

e_{ijkl}: random error (0, σ^2).

RESULTS AND DISCUSSION

Allelic profiles at microsatellite loci: The results obtained for number of alleles, their sizes and frequencies at egg production associated microsatellites loci have been presented in Table 2. Average number of alleles per locus was 3.7. The size of most frequent allele was 204 bp at locus MCW0145 and least frequent one as 173 bp at MCW0069. Two to three alleles at various polymorphic loci with an average of 2.41 alleles per locus were reported across the breeds (Deshmukh *et al.* 2015). Polymorphic loci having 2-6 alleles with an average of 3.5 alleles and 2-5 alleles with average of 4 alleles per locus were observed in selected strain of RIR chicken (Debnath 2016, Rahim *et al.* 2017). Thus, present findings were in close agreement

Table 1. Details of the primer sequences of microsatellite loci and their annealing temperature

Microsatellite	Chromosome location	Primer sequence (5'-3')	Annealing temperature (°C)
ADL0023	5	F: CTTCTATCCTGGGCTTCTGA R: CCTGGCTGTGTATGTGTTGC	61
ADL0158	E29	F: TGGCATGGTTGAGGAATACA R-TAGGTGCTGCACTGGAAATC	52
ADL0176	2	F: TTGTGGATTCTGGTGGTAGC R: TTCTCCCGTAACACTCGTCA	55
ADL0273	Z	F: GCCATACATGACAATAGAGG R: TGGTAGATGCTGAGAGGTGT	55
MCW0044	2	F: AGTCCGAGCTCTGCTCGCCTCATA R: ACAGTGGCTCAGTGGGAAGTGACC	63
MCW0069	26	F: GCACTCGAGAAAACTTCCTGCG R: ATTGCTTCAGCAAGCATGGGAGGA	55
MCW0103	3	F: AACTGCGTTGAGAGTGAATGC R: TTTCCTAACTGGATGCTTCTG	55
MCW0110	E48	F: CATCTGTGTTACTGTCACAG R: TCAGAGCAGTACGCCGTGGT	58
MCW0145	1	F: ACTTTATTCTCCAAATTTGGCT R: AAACACAATGGCAACGGAAC	55
MCW0 258	Z	F: TTCTTAGTCCTTGCCAGAGGC R: CTGCAGGAGGATGTGTCCTAG	55

Table 2. Number of alleles, molecular sizes and frequencies at egg production associated microsatellites loci in RIR chicken

Microsatellite	Chromosome location	No. of alleles	Allele code	Allele size (bp)	Allele frequency
ADL0023	5	3	A	190	0.1748
			B	178	0.3398
			C	172	0.4854
ADL00158	E29	4	A	212	0.0485
			B	200	0.2524
			C	194	0.6408
			D	182	0.0583
ADL0176	2	z7	A	220	0.0340
			B	215	0.2524
			C	210	0.0583
			D	205	0.2621
			E	200	0.0971
			F	195	0.2670
			G	190	0.0291
ADL0273	Z	3	A	150	0.0291
			B	147	0.5243
			C	144	0.4466
MCW0044	2	4	A	150	0.0388
			B	147	0.2621
			C	141	0.4466
			D	138	0.2525
MCW0069	26	5	A	173	0.0146
			B	169	0.1359
			C	165	0.1893
			D	161	0.1214
			E	157	0.5388
MCW0103	3	2	A	300	0.4854
			B	292	0.5146
MCW0110	E48	4	A	116	0.2670
			B	112	0.2718
			C	104	0.2087
			D	100	0.2525
MCW0145	1	2	A	220	0.0485
			B	204	0.9515
MCW0258	Z	3	A	170	0.5340
			B	158	0.4078
			C	146	0.0582
Mean ± SE		3.70±0.47			

to the earlier findings in chicken and demonstrated their applicability.

Population structure: Nei's heterozygosity of microsatellite (MS) loci was 0.55±0.06 ranging from 0.09 (MCW0145) to 0.78 (ADL0176). Similar estimates for different microsatellites were reported in various poultry breeds (Vijh and Tantai 2004, Debnath 2016). The studied population was investigated for it being in Hardy-Weinberg equilibrium by Chi-square and G square likelihood ratio tests. The results of Chi-square test and likelihood ratio tests revealed that the studied population was in Hardy-Weinberg disequilibrium at all ten MS loci which might be due to influence of external forces. Observed and expected heterozygosity were 0.1922±0.0687 and 0.5607±0.06, respectively with population in Hardy-Weinberg disequilibrium. It might be due to association of microsatellite loci with economic traits as the population was undergoing continuous selection for part-period egg production trait and was also small in size. Similar to present findings, observed and expected heterozygosities were reported in Ankaleshwar at ADL0023 as 0.333 and 0.804, at ADL0176 as 0.816 and 0.740 and at ADL0158 as 0.605 and 0.594, respectively (Pandey *et al.* 2005). There was a report of high observed and expected heterozygosities for ADL0023 (0.91 and 0.79), ADL0158 (0.92 and 0.72), ADL0176 (0.90 and 0.80), MCW0044 (0.27 and 0.49) and

MCW0110 (0.73 and 0.73) loci in Kadaknath and Aseel breeds of chicken (Chatterjee *et al.* 2010b). Earlier reports (El-sayed *et al*. 2011, Suh *et al*. 2014, Debnath *et al*. 2015b, Ramadan *et al*. 2024) also demonstrated similar estimates of observed and expected heterozygosities. The estimates were comparable to earlier reports in RIR chicken. Five out of 10 MS loci had polymorphic information content (PIC) value more than 50%. The estimated average PIC value was 0.498±0.06, which was ranging from 0.0880 (MCW0145) to 0.7489 (ADL0176), respectively and were in accordance with the earlier findings in RIR population (Vijh and Tantai 2004, Pandey *et al.* 2005), although a few contrary reports are also available for the studied MS loci (Chatterjee *et al*. 2008a, Chatterjee *et al*. 2010b, Deshmukh *et al.* 2015). The differences in PIC values might be due to variation in genetic architecture of population analyzed or due to loss/fixation of some of alleles during long-term selection.

Production performance: Least squares analysis of variance of body weight at 20 weeks of age (BW20) and various layer economic traits viz., age at sexual maturity (ASM), egg weight at 28 (EW28) and 40 (EW40) weeks of age and egg production up to 40 (EP40) weeks of age has been presented in Table 3. Sire had significant ($p<0.05$) effect on layer traits EW40 and EP40. Hatch had significant effect ($p<0.05$) on ASM and EW28. Least squares mean for BW20, ASM, EW28, EW40 and EP40 have been presented in Table 4. Overall, least-squares mean of ASM, EW28, EW40 and EP40 were 134.98±1.30 days, 42.54±0.32 g, 48.15±0.56 g and 121.47±2.44 eggs, respectively. Least-squares means of body weight revealed that birds of 1st hatch showed highest BW20 (1378.27±53.33g) followed by 3rd (1372.71±48.13 g), 4th (1347.96±32.32 g), 5th (1336.05±35.63 g) and 2nd (1280.58±54.93 g) hatch. Pullets of 5th hatch showed highest EW28 (44.84±0.66 g), while those of 2nd hatch showed highest EW40 (49.96±1.26g), and 1st hatch showed highest EP40 (127.92±5.55). Although, same management were practices followed in all the hatches, still the effect of hatch was significant ($p<0.05$). It might be due to micro-environmental variability as well as quick environmental fluctuations among the hatches which were beyond the human control. Similar findings were reported for significant hatch effect on early egg weights (Smith and Bohren 1975), on egg production (King and Henderson 1954) and on ASM in the coloured Punjab Broiler-II (PB2) dam line (Madapurada 2001, Nwague *et al.* 2007, Das 2013, Debnath *et al.* 2015a).

Genetic parameters of layer economic traits: Genetic parameters amongst BW20 and various layer economic traits have been presented in Table 5. Heritability estimate for BW20 was 0.38±0.48. Quite comparable heritability estimates with present investigation were reported by some of the researchers (Qadri *et al.* 2013, Rahim *et al.* 2017). However, some authors have reported high heritability for BW20 chicken (Jilani *et al.* 2005, Debnath 2016), which might be due to differences in the genetic stocks evaluated. Heritability estimates were high for ASM (0.49±0.48), EW40 (0.97±0.50) and EP40 (0.86±0.50), but low for EW28 (0.19±0.46). Similar heritability for ASM has been reported by earlier researchers also (Rahim 2015, Debnath 2016, Jayalaxmi *et al.* 2010). Lower heritability estimates indicated that it was difficult to improve this trait through genetic selection. However, contrary to the present findings, some researchers have reported lower heritability estimate for EW28, EW40 and EP40 (Anees *et al*. 2010, Rajkumar *et al*. 2011). Genetic and phenotypic correlations between layer traits and EP40 were negative except between BW20 and EP40, where it was low but positive. Genetic correlations (rG) of BW20 with EW28 and EW40 were positive, but negative with EP40. The rG

Table 3. Least squares analysis of variance of layer traits in Rhode Island Red chicken

Source of variation	df	Mean sum of squares				
		ASM	BW20	EW28	EW40	EP40
Sire	27	121.05	22286.35	7.13	19.82*	375.81*
Hatch	4	380.73*	11556.03	23.13**	20.98	143.70
Error/ Remainder	59	115.30	21695.30	6.22 (55)	10.17 (55)	203.91 (55)

df: Degrees of freedom, *$p<0.05$, **$p<0.01$, Figures within parentheses denote degrees of freedom

Table 4. Least squares mean ± S.E. of layer traits in Rhode Island Red chicken

Factors		N	Least squares mean ± standard errors				
			ASM (days)	BW20 (g)	EW28 (g)	EW40 (g)	EP40
Overall		91	134.98±1.30	1343.12±17.65	42.54±0.32 (87)	48.15±0.56 (87)	121.47±2.44 (87)
Hatch	1	12	140.75±3.89^{a}	1378.27±53.33	41.39±0.92^{b}	47.56±1.23	127.92±5.55
	2	9	132.54±4.01^{cd}	1280.58±54.93	42.71±0.94^{b}	49.96±1.26	123.16±5.66
	3	12	139.86±3.51^{ab}	1372.71±48.13	41.47±0.82^{b}	47.25±1.12	119.46±5.01
	4	28	134.77±2.36^{bc}	1347.96±32.32	42.29±0.567^{b}	46.74±0.81	119.64±3.60
	5	30	126.99±2.60^{d}	1336.05±35.63	44.84±0.66^{a} (26)	49.24±0.93 (26)	117.17±4.10 (26)

N: Number of observations, Means with same superscript in a column do not differ significantly ($p<0.05$), Figures within parentheses denote number of observations.

Table 5. Heritability, genetic and phenotypic correlations amongst various layer traits

Trait	ASM	BW20	EW28	EW40	EP40
ASM	0.49±0.48 (91)	-0.74±1.18 (91)	-0.49±1.27 (87)	0.34±0.59 (87)	-0.58±0.77 (87)
BW20	-0.26 (91)	0.38±0.48 (91)	0.59 ± 1.26 (87)	0.12±0.60 (87)	-0.06±0.62 (87)
EW28	0.005 (87)	0.16 (87)	0.19±0.46 (87)	0.20±0.77 (87)	-0.32±1.06 (87)
EW40	-0.06 (87)	0.14 (87)	0.46 (87)	0.97±0.50 (87)	-0.62±0.47 (87)
EP40	-0.28 (87)	0.07 (87)	-0.16 (87)	-0.05 (87)	0.86±0.50 (87)

Figures within parentheses denote number of observations

Table 6. Estimated mean sum of squares of layer economic traits under different microsatellite loci in Rhode Island Red chicken

Source of variation	Mean sum of squares					
	df	ASM	BW20	EW28	EW40	EP40
ADL0023	3	58.47	80830.27**	5.72	4.90	188.24
ADL0158	3	239.98	5697.87	5.20	10.57	144.30
ADL0176	8	117.34	20385.26	8.21	5.44	77.99
ADL0273	2	67.69	58488.71$	25.18*	14.31	2.36
MCW0044	3	133.52	17993.42	3.03	1.93	23.12
MCW0069	6	48.24	6015.21	6.03	13.92	286.92
MCW0103	1	34.45	20299.47	4.19	0.15	9407.69
MCW0110	8	96.61	24455.32	8.941	10.07	72.59
MCW0145	1	3.28	52603.44	0.06	13.69	11.20
MCW0258	4	37.68	20809.26	11.17	11.53	41.17

df: Degrees of freedom, $p<0.07, *p<0.05, **p<0.01

of ASM with BW20, EW28 and EP40 were negative, but positive with EW40. The rG of EW28 was positive with EW40, but negative with EP40. The rG of EW40 was negative with EP40. The phenotypic correlations (rP) of ASM with all other traits were negative except EW28. The rP of BW20 was positive with EW28, EW40 and EP40. The rP of EW28 was positive and high with EW40, but negative with EP40. Likewise, EW40 had negative rP with EP40. Similar negative genetic correlations of ASM with layer traits have also been reported earlier (Jayalaxmi *et al.* 2010, Qadri *et al.* 2013, Rahim 2015). A few earlier reports also demonstrated that BW20 had positive genetic correlations with EW28 and EW40 (Das *et al*. 2015), and negative with ASM and EP40 in selected strain of RIR chicken (Rahim 2015, Debnath 2016).

Association of microsatellite genotypes with layer economic traits: All the experimental birds were genotyped for 10 microsatellites were found polymorphic. Least squares analysis of variance of layer economic traits to determine the effects of MS genotypes taking it as independent factor in the model and MS genotype-wise LS means of various traits are given in Table 6 and Table 7, respectively. Genotypes at two MS loci (ADL0023 and ADL0273) revealed significant (p<0.01 and p<0.07, respectively) effects on BW20. AA genotype at ADL0023 had revealed highest body weight (1496.34±45.52 g) at BW20 which was statistically higher than those pullets having genotypes BB (1295.52±36.01g), CC (1325.76±32.89 g) or AB (1334.04±78.34 g). Significant effect of genotypes at ADL0023 on BW20 had been reported by earlier researchers (Chatterjee *et al.* 2008b, Rahim *et al.* 2017, Debnath *et al.* 2019). ADL0273 genotypes had significant effect on BW20 (p<0.07) and EW28 (p<0.05). Pullets with AA genotype at this locus showed highest BW20 as 1530.30±77.34 g which was statistically higher than those pullets having BB (1365.01±28.79 g) and CC (1305.50±36.95 g). AA genotyped pullets revealed highest EW28 (45.01±1.50 g) which was statistically different than CC (41.16±0.69 g) genotyped birds, but did not differ from BB (43.26±0.58 g) genotyped birds. Similar to the present findings, earlier researchers had reported significant effect ADL0273 genotypes on ASM, BW20 and EW28 (Arya 2012, Radwan *et al*. 2014, Das *et al*. 2015, Debnath *et al*. 2015b, Thamer and Noori 2022). The rest of the MS-genotypes at other loci did not differ significantly for any of the layer economic traits. The non-significant effect of MS-genotypes at these loci was supported by previous reports (Chatterjee *et al.* 2008a, Das *et al*. 2015). Contrary to the present findings some of the reports suggested significant effect of genotypes at ADL0158, ADL0176, MCW0069,

Table 7. Least squares mean ± S.E of layer traits for different genotypes at microsatellite loci in Rhode Island Red chicken

Microsatellite	Genotype	Least squares mean ± standard error				
		ASM (days) (n=110)	BW20 (g) (n=110)	EW28 (g) (n=108)	EW40 (g) (n=103)	EP40 (n=110)
ADL0023	AA	132.832 ± 3.63	1496.34[a] ± 45.52	42.18 ± 0.89	48.08 ± 1.20	118.07 ± 5.42
	BB	136.63 ± 2.88	1295.52[b] ± 36.01	42.54 ± 0.68	47.39 ± 0.94	124.39 ± 4.27
	CC	134.62 ± 2.63	1325.76[b] ± 32.89	42.77 ± 0.63	48.74 ± 0.93	126.14 ± 4.23
ADL0158	AB	141.50 ± 6.25	1334.04[b] ± 78.34	40.04 ± 1.47	48.81 ± 2.11	134.72 ± 9.45
	BB	139.76 ± 3.97	1313.12 ± 55.98	42.09 ± .95	47.85 ± 1.25	121.65 ± 5.71
	CC	135.66 ± 2.30	1351.37 ± 30.93	42.56 ± .52	47.90 ± .81	124.06 ± 3.69
	DD	134.36 ± 4.99	1398.58 ± 70.83	44.16 ± 1.21	48.89 ± 1.64	124.51 ± 7.51
ADL0176	AB	125.74 ± 4.69	1326.84 ± 66.44	41.27 ± 1.13	50.37 ± 1.46	132.48 ± 6.68
	BB	137.20 ± 3.23	1276.81 ± 44.50	43.26 ± 0.76	47.63 ± 1.04	120.52 ± 4.86
	DD	135.47 ± 3.69	1435.39 ± 50.86	44.01 ± 0.87	48.86 ± 1.19	127.38 ± 5.54
	FF	132.79 ± 5.22	1285.72 ± 71.94	40.17 ± 1.21	47.38 ± 1.64	123.65 ± 7.549
	AB	120.25 ± 9.27	1396.75 ± 127.90	40.67 ± 2.16	52.57 ± 2.85	112.61 ± 13.06
	AD	136.68 ± 8.68	1490.25 ± 119.72	43.67 ± 2.02	48.50 ± 2.73	126.17 ± 12.53
	CD	134.81 ± 7.30	1363.10 ± 100.67	43.75 ± 1.70	49.81 ± 2.26	127.69 ± 10.39
	CE	138.76 ± 5.95	1375.91 ± 82.01	41.79 ± 1.39	47.37 ± 1.85	133.77 ± 8.52
	EF	125.40 ± 8.30	1154.59 ± 114.42	41.31 ± 1.94	46.83 ± 2.58	122.25 ± 11.84
	EG	142.39 ± 6.23	1302.03 ± 85.90	40.65 ± 1.45	48.25 ± 1.92	122.92 ± 8.85
ADL0273	AA	137.60 ± 5.85	1530.30[a] ± 77.34	45.01[a] ± 1.50	52.41 ± 2.31	124.32 ± 10.63
	BB	136.59 ± 2.17	1365.01[b] ± 28.79	43.26[a] ± 0.58	48.33 ± 0.97	124.26 ± 4.36
	CC	132.87 ± 2.79	1305.50[c] ± 36.95	41.16[b] ± 0.69	47.76 ± 1.08	125.01 ± 4.88
MCW0044	AA	139.43 ± 7.51	1373.84 ± 103.81	43.78 ± 1.81	47.28 ± 2.29	119.00 ± 10.42
	BB	136.93 ± 3.27	1293.06 ± 44.97	41.91 ± 0.79	47.99 ± 1.06	124.58 ± 4.85
	CC	132.84 ± 2.63	1371.75 ± 36.10	42.58 ± 0.63	48.48 ± 0.87	125.23 ± 4.012
	DD	139.11 ± 3.76	1364.84 ± 51.83	43.03 ± 0.90	47.78 ± 1.18	123.58 ± 5.41
MCW0069	CC	134.37 ± 5.17	1285.23 ± 71.38	43.81 ± 1.19	48.28 ± 1.57	134.50 ± 7.08
	DD	133.63 ± 4.96	1332.67 ± 68.52	41.85 ± 1.16	50.83 ± 1.60	127.40 ± 7.23
	EE	134.90 ± 2.79	1366.64 ± 38.53	42.80 ± 0.66	48.42 ± 0.90	118.57 ± 4.06
	AE	142.48 ± 7.04	1414.44 ± 97.26	39.40 ± 1.92	42.22 ± 2.36	133.09 ± 10.65
	BD	134.30 ± 5.50	1359.18 ± 76.06	42.12 ± 1.28	48.88 ± 1.62	135.38 ± 7.29
	BE	132.58 ± 3.19	1347.84 ± 44.17	41.90 ± 0.74	47.54 ± 1.02	121.75 ± 4.59
	CE	137.83 ± 3.53	1325.33 ± 48.84	42.87 ± 0.81	48.28 ± 1.06	126.98 ± 4.77
MCW0103	AA	134.39 ± 1.84	1366.63 ± 24.88	42.20 ± 0.43	48.18 ± 0.61	125.99 ± 2.78
	BB	135.91 ± 1.78	1329.67 ± 24.06	42.73 ± 0.41	48.18 ± 0.58	123.32 ± 2.69
MCW0110	AA	136.66 ± 3.66	1301.64 ± 48.78	42.89 ± 0.84	48.74 ± 1.14	123.16 ± 5.41
	BB	136.65 ± 3.75	1265.68 ± 50.04	41.37 ± 0.89	46.89 ± 1.23	125.93 ± 5.83
	CC	136.93 ± 6.88	1336.34 ± 92.10	42.01 ± 1.57	46.61 ± 2.02	120.81 ± 9.62
	DD	139.42 ± 4.90	1409.39 ± 65.53	44.05 ± 1.13	50.80 ± 1.47	122.14 ± 6.97
	AC	135.38 ± 3.21	1382.04 ± 42.71	41.95 ± 0.78	48.57 ± 1.05	124.46 ± 4.99
	AD	134.43 ± 5.89	1402.08 ± 78.85	40.80 ± 1.34	50.72 ±1.74	134.03 ± 8.28
	BC	121.14 ± 7.32	1532.04 ± 98.12	45.80 ± 2.07	49.18 ± 2.67	127.92 ± 12.69
	BD	131.87 ± 3.23	1362.80 ± 43.04	43.68 ± 0.76	47.47 ± 1.026	122.01 ± 4.84
	CD	135.61 ± 6.41	1407.59 ± 85.91	43.51 ± 1.46	48.85 ± 1.89	131.12 ± 8.97
MCW0145	BB	135.12 ± 1.83	1356.02 ± 24.24	42.49 ± 0.42	48.03 ± 0.72	124.69 ± 3.28
	AB	135.88 ± 3.69	1260.51 ± 49.40	42.39 ± 0.87	49.70 ± 1.22	123.18 ± 5.60
MCW0258	AA	136.50 ± 3.46	1303.88 ± 46.46	41.70 ± 0.79	47.55 ± 1.07	124.37 ± 4.88
	BB	132.67 ± 3.64	1358.95 ± 48.84	42.37 ± 0.84	48.29 ± 1.13	125.58 ± 5.18
	CC	130.58 ± 11.37	1596.32 ± 152.53	48.87 ± 2.57	53.71 ± 3.35	118.54 ± 15.62
	AB	136.53 ± 4.27	1361.60 ± 57.31	43.15 ± 0.97	49.06 ± 1.35	125.94 ± 6.22
	BC	135.61 ± 7.01	1358.21 ± 94.03	41.76 ± 1.59	46.04 ± 2.06	118.57 ± 9.61

N: Number of observations, Means with same superscript in a column do not differ significantly ($p<0.05$ and $p<0.07$), Figures within parentheses denote number of observations.

MCW0103, MCW0110, MCW0145 and MCW0258 on egg production and egg weight (Roushdy *et al.* 2008, Rahim *et al*. 2017).

To conclude, age at sexual maturity in the studied population followed similar trend over the generations. Body weight at 20-weeks of age revealed low but positive phenotypic correlations with egg production up to 40 weeks. Owing to high association of AA genotype at ADL0023 MS loci with body weight at 20 weeks and AA genotype at ADL0273 with body weight at 20 weeks and egg weight at 28 weeks, these genotypes may be considered promising markers for genetic improvement of layer traits in poultry.

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COMPLIANCE WITH ETHICAL STANDARDS

The samplings from experimental birds were done in accordance with the ethical standards approved by Institute Animal Ethical Committee.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

20th Livestock Census. 2019. Department of Animal Husbandry, Dairying & Fisheries Annual Report, Ministry of Agriculture, Govt. of India, New Delhi.

Abasht B, Dekkers J C M and Lamont S J. 2006b. Review of Quantitative trait loci identified in the chicken. *Poultry Science* **85**: 2079-96.

Abasht B, Pitel F, Lagarrigue S, Duval E L, Roy P L, Demeure O, Vignoles F, Simon J, Cogburn L, Aggrey S, Vignal A and Douaire M. 2006a. Fatness QTL on chicken chromosome 5 and interaction with sex. *Genetics Selection Evolution* **38**: 297-311.

Ahlawat S P S, Sunder J, Kundu A, Chatterjee R N, Rai R B, Kumar B, Senani S, Saha S K and Yadav S P. 2004. Use of RAPD-PCR for genetic analysis of Nicobari fowl of Andamans. *British Poultry Science* **45**: 1-7.

Anees C, Veeramani P, Narayanankutty K, Jacob A and Riyas M A. 2010. Estimation of genetic and phenotypic parameters of economic traits in White Leghorn. *Indian Journal of Poultry Science* **45**: 14-17.

Arya R. 2012. Short tandem repeats (STR) polymorphism in egg-type chicken and its association with egg production traits. Ph.D. Thesis, Indian Veterinary Research Institute, Izatnagar, Deemed University, India.

Bakare I O, Ilori B M, Wheto M, Egbeyale L T, Sanda A J and Olowofeso O. 2021. Genetic diversity and gene flow among three chicken populations in Nigeria using microsatellite markers. *Agriculturae Conspectus Scientificus* **86**(2): 173-181.

Basic Animal Husbandry and Fisheries Statistics. 2019. Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture, Government of India, Krishi Bhawan, New Delhi.

Botstein D, White R L, Skolnick M and Davis R W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics* **32**: 314-31.

Chatterjee R N, Sharma R P, Mishra A, Dange M and Bhattacharya T K. 2008a. Variability of microsatellites and their association with egg production traits in chicken. *International Journal of Poultry Science* **7**: 77-80.

Chatterjee R N, Sharma R P, Mishra A, Dange M and Bhattacharya T K. 2008b. Association of microsatellites with growth and immunocompetence traits in crossbred layer chicken. *Journal of Poultry Science* **45**: 186-91.

Chatterjee R N, Sharma R P, Reddy B L N, Niranjan M and Mishra S K. 2007. Genetic analysis of highly inbred chicken using RAPD-PCR and immunocompetence. *International Journal of Poultry Science* **6**: 967-72.

Chatterjee R N, Niranjan M, Sharma R P, Dange M and Bhattacharya T K. 2010b. Estimation of genetic heterogeneity of chicken germplasm being used for development of rural varieties utilizing DNA markers. *Journal of Genetics* **89**: 33-37.

Chatterjee R N, Sharma R P, Bhattacharya T K, Niranjan M and Reddy B L. 2010a. Microsatellite variability and its relationship with growth, egg production, and immunocompetence traits in chickens. *Biochemical Genetics* **48**: 71-82.

Das A K. 2013. Microsatellite Polymorphism, Immunocompetence Profile and Performance Evaluation of Rhode Island Red chicken and its crosses. Ph.D. Thesis, Indian Veterinary Research Institute, Izatnagar, Deemed University, India. pp: 1-177.

Das A K, Kumar S and Rahim A. 2015. Estimating microsatellite-based genetic diversity in Rhode Island Red chicken. *Iranian Journal of Veterinary Research* **16**(3): 274–277.

Debnath J, Kumar S, Bhanja S K, Rahim A and Yadav R. 2015a. Factors influencing early layer economic traits in Rhode Island Red chicken. *Journal of Animal Research* **5**(4): 915-19.

Debnath J, Kumar S, Yadav R and Rahim A. 2015b. Microsatellite genotypes of sire influence early layer economic traits and mortality in Rhode Island Red chicken. *Indian Journal of Poultry Science* **50**(3): 248-53.

Debnath J, Kumar S, Das A K and Rahim A. 2019. Association of microsatellites with pre-housing body weights and age at sexual maturity of Rhode Island Red chicken. *Indian Journal of Animal Science* **89**(10): 1118-22.

Debnath J. 2016. Microsatellites profiling and their association with layer economic traits in Rhode Island Red chicken. Ph.D. Thesis, Indian Veterinary Research Institute, Izatnagar, Deemed University, India. pp: 1-142.

Deshmukh B, Kumar D, Kashyap N and Sharma D. 2015. Study of genetic polymorphism of various chicken breeds using microsatellite markers. *Indian Journal of Animal Research* **49**: 1-7.

El-sayed M A, Roushdy K H, Galal A and El-attar A H. 2011. *Genetic differentiation of two Egyptian chicken breeds using 15 microsatellite markers*. Proceedings of the 3rd International

Conference on Genetic Engineering and Applications. pp: 149-61.

Jayalaxmi P, Gupta R B, Chatterjee R N, Sharma R P and Reddy R V. 2010. Genetic analysis of growth and production traits in IWK strain of White Leghorn. *Indian Journal of Poultry Science* **45**: 123-26.

Jilani M H, Harpal S and Singh C B. 2005. Performance evaluation and selection indices in a strain of Rhode Island Red. *Indian Journal of Poultry Science* **40**: 278-81.

King S C and Henderson C R. 1954. Variance components analysis in heritability studies. *Poultry Science* **33**(1): 147–154.

Madapurada A. 2001. Evaluation for egg production and allied traits in colored broiler breeder dam line. M.V.Sc. Thesis, University of Agricultural Sciences, Bangalore, Karnataka, India.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**: 583-90.

Nwague B I, Olorunju S A S, Oni O O, Eduvie L O, Adeyinka I A, Sekoni A A and Abeke F O. 2007. Response of egg number to selection in Rhode Island chickens selected for part period egg production. *International Journal of Poultry Science* **6**(1): 18-22.

Pandey A K, Kumar D, Sharma R, Sharma U, Vijh R K and Ahlawat S P S. 2005. Population structure and genetic bottleneck analysis of Ankleshwar poultry breed by microsatellite markers. *Asian-Australasian Journal of Animal Science* **18**(7): 915-21.

Qadri F S, Savaliya F P, Patel A B, Joshi R S, Hirani N D and Patil S S. 2013. Genetic study on important economic traits in two strains of White Leghorn chicken. *Indian Journal of Poultry Science* **48**: 149-53.

Radwan L M, El-Dlebshany A E and El-Denary M E. 2014. Microsatellite genetic differentiation analysis and organic matrix of eggshell in the 16th generation of chickens selected for egg production traits. *Egyptian Journal of Animal Production* **51**(1): 41-47.

Rahim A. 2015. Microsatellite, immunocompetence, and candidate gene expression profiling of Rhode Island Red chicken and association of microsatellite alleles and immunocompetence traits with layer economic traits. Ph.D. Thesis, Indian Veterinary Research Institute, Izatnagar, Deemed University, India. pp: 1–250.

Rahim A, Kumar S, Yadav R, Debnath J and Krishnan J. 2017. Genetic variability determination in a long-term selected Rhode Island Red chicken strain using microsatellite markers. *Veterinarski Arhiv* **8**(4): 511-22.

Rahim A, Kumar S, Das A K, Debnath J, Yadav R and Krishnan J. 2023. Association of microsatellite genotypes with layer economic traits in a selected strain of Rhode Island Red chicken. *The Indian Journal of Animal Sciences* **93**(12): 1193-98.

Rajkumar U, Reddy B L N, Padhi M K, Haunshi S, Niranjan M, Bhattacharya T K and Chatterjee R N. 2011. Inheritance of growth and production traits in sex-linked dwarf chicken of laying cycle in 64 weeks. *Indian Journal of Poultry Science* **48**: 143-47.

Ramadan G S, Stino F K, El-Komy E M, El Sabry M I, Rashed O S and Ghaly M M. 2024. Analysis of the genetic diversity in the cairo-mix broiler chicken using microsatellite and start codon targeted markers. *Egyptian Journal of Veterinary Sciences* **1-12**.

Roushdy K, El-Dein A Z, Fathi M M, Ali U M and Assy H M. 2008. Microsatellite genetic differentiation analysis of two local chicken breeds compared with foreign Hy-line strain. *International Journal of Poultry Science* **7**(11): 1045–53.

Sambrook J and Russell D W. 2001. *Molecular Cloning: A Laboratory Manual.* 3rd Ed. Cold Spring Harbor Laboratory Press, New York, NY.

Smith K P and Bohren B B. 1975. Age of pullet effects on hatching time, egg weight, and hatchability. *Poultry Science* **54**(4): 959–63.

Suh S, Sharma A, Lee S, Cho C Y, Kim J H, Choi S B, Kim H, Seong H H, Yeon S H, Kim D H and Ko Y G. 2014. Genetic diversity and relationships of Korean chicken breeds based on 30 microsatellite markers. *Asian-Australasian Journal of Animal Science* **27**(10): 1399-405.

Tautz D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research* **17**: 6463-71.

Thamer M A and Noori A A. 2022. Use of microsatellite marker mcw0330 for evaluating the productive performance of Isa brown egg laying hens. *Indian Journal of Ecology* **18**: 384-87.

Vijh R K and Tantai M S. 2004. Assignment of individuals to four poultry breeds of India using multilocus genotypes. *Indian Journal of Animal Science* **74**: 73–76.

Vikki J. 2012. *QTL for egg quality*. Proceedings of the 6th European Poultry Genetics Symposium. pp: 38–41.

Yeh F C, Yang R C and Boyle T. 1999. POPGENE version 1.31: Microsoft Windows-based freeware for population genetic analysis, quick user guide. Centre for International Forestry Research, University of Alberta, Edmonton, Alberta, Canada. Website: [http://ualberta.ca/~fyeh/popgene.html].