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Association of microsatellites with pre-housing body weights and age at sexual maturity of Rhode Island Red chicken#

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ABSTRACT

Present study was aimed to investigate microsatellites for their association with pre-housing body weights and age at sexual maturity of RIR chicken. Genomic DNA samples were isolated from 114 birds maintained at institute. PCR amplified products of selected microsatellite loci were separated on 3.4% MetaPhore™ agarose gel and their sizes were determined by Quantity One software on GelDoc system. Locus specific alleles were identified according to their sizes, and their association with the quantitative traits was assessed by least squares analysis of variance. The analysis revealed significant association of microsatellite MCW0069 locus with chick weight, ADL0158 and MCW0258 loci with eighth week body weight (BW8), MCW0103 locus with BW16, ADL0158 locus with BW20, ADL0273 and MCW0103 loci with age at sexual maturity (ASM). The highest chick weight estimates were found in AB (183:174 bp) genotype of MCW0069 locus, whereas EE (189:189 bp) and BE (219:189 bp) genotypes of ADL0158, AB (280:273 bp) and CC (267:267 bp) genotypes of MCW0103, CD (107:102 bp) genotype of MCW0110, AE (216:147 bp) genotype of MCW0258 demonstrated the highest pre-housing body weight estimates. AB (160:147 bp) genotype of ADL0273 locus demonstrated the least age at sexual maturity (127.39±4.23 days) followed by its BB (147:147 bp) genotype. CC genotype of MCW0103 also had the least ASM (132.46±2.46 days) among its other genotypes and was better than BB genotype of ADL0273 locus. These findings suggest faster genetic progress in RIR chicken line by adapting microsatellite genotype based selection.

Key words: Age at sexual maturity, Microsatellites, Pre-housing body weights, RIR Chicken

Microsatellites are tandem repeats of 1–6 bases, numerous, randomly distributed in the genome, polymorphic with co-dominant inheritance, widely used for genetic heterogeneity studies and provide a powerful tool for marker-assisted selection (MAS) of polygenic traits or QTL (quantitative trait loci) research (Das *et al.* 2015a). Several microsatellites have been reported for their association with some important economic traits like growth and egg production in chicken. Breeders exploit phenotypic variability in selection program for developing highly productive chicken genotypes for more egg and meat production. The ICAR-Central Avian Research Institute has developed and improved a selected line of RIR chicken population covering 29 generations of selection based on part-period egg production (Das *et al.* 2016), however the progress in response to selection has been slowing down in the last few generations probably due to reduction in genetic variability (Das *et al.* 2015a). Using genomic selection further faster genetic progress may be possible, which may have impact on layer breeding in future, and for which the microsatellites strive for its exploitation more widely. Present study was carried out investigating if there be any association between the microsatellites and few polygenic traits of economic importance like pre-housing body weights and age at sexual maturity in this selected line of RIR chicken.

MATERIALS AND METHODS

Straight run chicks (286), progeny of 11 sires, belonging to 30th generation of a selected line of RIR chicken, were produced through molecular breeding of RIR chicken based on the genotypes at ADL0176 microsatellite locus (Debnath *et al.* 2017). All straight run chicks were maintained at the experimental layer farm of this institute under standard brooding, housing and *ad lib.* feeding management. Standard vaccination practices were being followed. Females (114) were chosen randomly for this study. Genomic DNA was extracted from 0.1 ml venous blood of each experimental bird, followed by its quality

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Table 1. Estimated P values of pre-housing body weights and age at sexual maturity under different microsatellite loci of RIR chicken

Source of variation	df	P value					
		CW	BW8	BW12	BW16	BW20	ASM
ADL0023	4	0.1575	0.2351	0.1463	0.2219	0.1847	0.3375
ADL0158	6	0.1635	0.0004***	0.1702	0.0589	0.0304*	0.2484
ADL0273	3	0.9722	0.6366	0.7289	0.7549	0.9373	0.0275*
MCW0069	5	0.0216*	0.5511	0.3829	0.4399	0.1667	0.2084
MCW0103	4	0.4744	0.0738	0.1158	0.0500*	0.0699	0.018*
MCW0110	10	0.6449	0.1599	0.2750	0.0773	0.3849	0.2435
MCW0145	4	0.4535	0.7765	0.6859	0.5827	0.7516	0.9597
MCW0258	5	0.8739	0.0472*	0.3818	0.3428	0.1340	0.5612

*P≤0.05, ***P≤0.001; df, degree of freedom; CW, day-old chick weight; BW, body weights at different weeks of age; ASM, age at sexual maturity. Number of observation is 114 for each trait under each microsatellite.

checking on 0.7% horizontal submarine agarose gel electrophoresis, purity checking and quantity determination using NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies Inc., USA) (Debnath *et al.* 2017, Das *et al* 2015a,b). Samples showing intact DNA band and optical density ratio (260 nm : 280 nm) between 1.7 and 1.9 were used in subsequent experiments and PCR ready DNA samples were prepared at a final concentration of 50 ng/µl (Debnath *et al.* 2017). Eight microsatellite markers reported elsewhere for having association with various economic traits in different chicken breeds were identified from the published literatures (Das *et al.* 2016, Radwan *et al*. 2014, Chatterjee *et al*. 2010, Chatterjee *et al*. 2008) and their forward and reverse primers were procured from M/S Xcelris Genomics Labs Ltd., Ahmedabad (India). Annealing temperature for each of the primer pairs was optimized and PCR amplifications of the DNA samples were carried out for each microsatellite marker as described earlier (Debnath *et al*. 2017). The molecular sizes of amplified products were adjudged for their probable sizes through 2% horizontal agarose gel electrophoresis. The microsatellite alleles were then identified by running the amplified products on horizontal MetaPhore™ agarose gel electrophoresis (3.4% MAGE) (Debnath *et al*. 2017). 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) and the observed alleles with its probable genotypes in each sample at each microsatellite locus were recorded (Debnath *et al*. 2017). Locus specific alleles at each microsatellite locus were identified according to their molecular sizes (Das *et al.* 2015 ab) and nomenclatured with alphabet A to E in ascending order of their molecular sizes.

Data were recorded on pre-housing body weights (in g) of each bird, i.e. day-old chick weight (CW) and body weights at 8 (BW8), 12 (BW12), 16 (BW16) and 20 (BW20) weeks of age at empty stomach with help of digital weigh balance and the first egg laying age taken as age at sexual maturity (ASM, in days).

The data were analyzed for assessing their association with microsatellite genotypes by least squares analysis of variance using JMP 9.0.0 statistical program package (SAS, 2010) incorporating microsatellite locus as fixed effect in the linear 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 σ^2. Least significant difference (LSD) test at the 5% level of probability of significance was employed for assessing least significant differences among the least squares means under the genotypes of each microsatellite locus.

RESULTS AND DISCUSSION

The least squares analysis of variance revealed that some microsatellites had significant (P≤0.05) association with few pre-housing body weight traits and age at sexual maturity of RIR chicken (Table 1). Day-old chick weight (CW) was found significantly associated with microsatellite genotypes of MCW0069 locus. Similarly, body weight at eighth week of age (BW8) was significantly influenced by microsatellites ADL0158 and MCW0258; 16^{th} week body weight (BW16) by MCW0103; 20^{th} week body weight (BW20) by ADL0158 and age at sexual maturity (ASM) by ADL0273 and MCW0103 microsatellites (Table 1). In agreement to these findings, earlier few researchers also reported significant association of some microsatellites with age at sexual maturity (Wardecka *et al.* 2002, Chatterjee *et al.* 2008, Chatterjee *et al.* 2010, Radwan *et al*. 2014, Das *et al*. 2016), chick weight (Chatterjee *et al.* 2008, Chatterjee *et al.* 2010, Yadav *et al.* 2015) and pre-housing body weights (Wardecka *et al.* 2002, Sewalem *et al.* 2002, Chatterjee *et al.* 2008, Chatterjee *et al.* 2010, Yadav *et al.* 2015) in different chicken genotypes, thus paving way to microsatellite marker assisted selection of these traits in molecular breeding program for achieving further genetic progress in these chicken lines/strains while phenotypic variability gets exhausted.

The LSD test (Table 2) demonstrated that the eighth week

Table 2. Estimated least squares means of pre-housing body weights (BW) and age at sexual maturity (ASM) under different microsatellite genotypes of RIR chicken

Microsatellite loci	Microsatellite genotypes		Least squares means±standard errors					
	Code	allele (bp):allele (bp)	CW (g)	BW8 (g)	BW12 (g)	BW16 (g)	BW20 (g)	ASM (days)
ADL0023	AD	232:177	29.30±2.08	511.45±44.56	791.04±78.60	1155.03±104.73	1421.67±109.76	127.67±6.14
	BE	218:168	35.98±2.09	432.69±44.82	814.28±79.01	1160.57±105.34	1442.71±110.39	144.87±6.18
	CC	186:186	32.90±2.03	529.15±43.54	896.69±76.75	1245.57±102.33	1439.94±107.24	132.06±6.00
	DD	177:177	34.43±0.86	544.05±18.53	946.06±32.67	1322.25±43.56	1565.96±45.65	137.69±2.55
	EE	168:168	34.82±1.05	519.08±22.53	962.19±39.72	1353.34±52.95	1638.17±55.49	138.88±3.10
ADL0158	AD	247:196	32.70±2.02	507.78±36.37^{bc}	829.19±71.57	1328.76±92.19^{b}	1537.55±96.60^{bc}	131.29±5.52
	BD	219:196	33.65±1.83	401.69±32.92^{d}	867.59±64.77	1176.12±83.43^{c}	1443.63±87.42^{c}	141.82±5.00
	BE	219:189	36.23±2.20	567.71±39.54^{a}	967.04±77.81	1320.18±100.22^{b}	1617.84±105.02^{ab}	142.98±6.00
	CE	207:189	33.91±1.26	516.74±22.76^{ab}	924.83±44.79	1255.49±57.70^{bc}	1529.77±60.46^{bc}	141.39±3.46
	DD	196:196	34.03±0.89	547.58±16.03^{a}	941.89±31.54	1327.60±40.63^{b}	1567.65±42.58^{b}	133.72±2.43
	DE	196:189	32.65±32.65	439.72±50.51^{cd}	871.38±99.40	1251.46±128.03^{bc}	1564.16±134.16^{b}	150.93±7.67
	EE	189:189	37.43±1.42	550.74±25.56^{a}	1022.23±50.29	1431.53±64.78^{a}	1739.40±67.88^{a}	139.31±3.88
ADL0273	AB	160:147	33.99±1.55	562.59±31.94	980.83±56.84	1374.47±75.54	1597.10±79.77	127.39±4.23^{a}
	AC	160:141	32.99±3.33	521.15±68.54	935.02±121.98	1233.67±162.09	1547.15±171.18	148.55±9.06^{ab}
	BB	147:147	34.34±0.74	524.03±15.15	927.09±26.96	1303.85±35.82	1564.59±37.83	137.50±2.00^{ab}
	CC	141:141	33.97±2.11	504.05±43.37	880.83±77.19	1269.19±102.57	1513.14±108.33	146.09±5.74^{b}
MCW0069	AB	183:174	45.08±3.24^{a}	582.90±70.77	988.85±125.19	1317.33±167.03	1445.44±172.32	124.86±9.63
	AC	183:165	33.73±1.21^{b}	527.11±26.41	860.93±46.71	1248.78±62.32	1522.97±64.29	142.19±3.59
	AD	183:158	36.46±2.21^{ab}	493.14±48.20	865.31±85.25	1186.28±113.75	1444.44±117.35	147.24±6.55
	BC	174:165	31.10±1.71^{b}	483.20±37.28	870.81±65.94	1210.46±87.98	1406.83±90.77	133.02±5.07
	BD	174:158	33.73±1.31^{b}	539.96±28.57	969.25±50.53	1352.45±67.42	1609.36±69.56	138.43±3.89
	CC	165:165	34.67±0.96^{b}	517.17±20.97	935.44±37.08	1315.08±49.48	1573.32±51.05	136.04±2.85
MCW0103	AA	280:280	30.35±2.79	529.76±56.32^{ab}	836.04±99.98	1240.99±130.87^{ab}	1476.90±138.58^{b}	136.31±7.61^{ab}
	AB	280:273	34.97±1.27	543.74±25.61^{a}	965.98±45.47	1397.05±59.51^{a}	1708.72±63.02^{a}	139.26±3.46^{ab}
	BB	273:273	34.22±1.03	503.27±20.69^{b}	884.88±36.72	1227.05±48.06^{b}	1534.82±50.90^{b}	143.19±2.80^{b}
	BC	273:267	32.58±1.73	455.32±34.86^{c}	833.75±61.88	1199.47±80.99^{b}	1456.28±85.77^{b}	139.12±4.71^{ab}
	CC	267:267	34.14±0.90	541.38±18.19^{a}	950.50±32.28	1323.16±42.26^{a}	1525.25±44.75^{b}	132.46±2.46^{a}
MCW0110	AC	119:107	32.91±1.50	517.23±29.95	927.26±53.89	1277.12±69.60^{cd}	1564.69±75.84	144.43±4.18
	AD	119:102	34.15±1.93	459.69±38.43	957.99±69.15	1328.13±89.30^{bcd}	1610.66±97.32	134.48±5.36
	BB	113:113	34.17±2.71	446.54±54.10	894.69±97.33	1365.07±125.70^{bcd}	1667.86±136.98	137.06±7.54
	BC	113:107	32.29±2.10	517.39±41.85	924.25±75.29	1236.01±97.23^{d}	1516.13±105.96	142.71±5.36
	BD	113:102	35.85±1.29	507.51±25.72	936.22±46.28	1275.83±59.77^{d}	1557.27±65.13	137.89±5.83
	BE	113:99	37.37±2.87	576.27±57.26	1116.9±103.01	1473.06±133.04^{b}	1659.65±144.97	127.69±3.59
	CC	107:107	33.36±1.19	523.53±23.77	875.75±42.77	1269.27±55.24^{d}	1564.45±60.19	140.17±7.98
	CD	107:102	37.36±2.90	656.65±57.80	1176.2±103.98	1737.42±134.29^{a}	1902.91±146.34	125.65±3.31
	CE	107:99	35.69±1.42	563.35±28.38	976.71±51.06	1377.65±65.95^{bcd}	1539.72±71.86	129.55±8.06
	DD	102:102	33.24±1.32	519.35±26.47	891.85±47.63	1253.25±61.51^{d}	1476.45±67.03	133.81±3.69
	EE	99:99	35.42±2.08	538.69±41.50	962.56±74.66	1413.84±96.42^{bc}	1679.46±105.07	137.77±5.78

(*Table 2. Contd...*)

Microsatellite loci	Microsatellite genotypes		Least squares means±standard errors					
	Code	allele (bp):allele (bp)	CW (g)	BW8 (g)	BW12 (g)	BW16 (g)	BW20 (g)	ASM (days)
MCW0145	AB	226:209	34.29±1.51	526.08±31.61	916.33±55.97	1256.31±74.09	1577.99±78.32	135.80±4.39
	AC	226:203	35.11±1.26	534.72±26.54	940.61±46.99	1366.35±62.20	1613.58±65.75	137.73±3.69
	BB	209:209	35.07±0.96	517.42±20.20	934.01±35.77	1298.37±47.35	1543.64±50.05	137.81±2.81
	BC	209:203	33.77±1.80	569.19±37.65	1015.5±66.66	1400.06±88.23	1645.52±93.27	133.92±5.23
	CC	203 : 203	33.15±0.96	526.16±20.16	911.85±35.70	1300.31±47.26	1545.37±49.96	137.11±2.80
MCW0258	AC	216:168	35.55±1.73	502.88±33.25^{b}	914.01±59.27	1295.39±82.53	1554.22±84.62	137.70±4.96
	AD	216:161	35.60±1.29	513.10±24.78^{b}	947.29±44.18	1320.86±61.51	1587.28±63.07	139.53±3.70
	AE	216:147	33.46±2.11	583.73±40.46^{a}	972.15±72.12	1403.66±100.42	1645.92±102.97	138.38±6.04
	BE	201:147	33.21±2.09	420.81±40.18^{c}	812.72±71.63	1122.58±99.74	1323.35±102.27	144.31±6.00
	DD	161:167	33.91±1.75	524.88±33.57^{ab}	869.09±59.85	1245.54±83.33	1475.02±85.45	137.32±5.01
	EE	147:147	33.09±1.31	551.43±25.22^{a}	946.70±44.96	1324.93±62.60	1589.65±64.19	132.80±3.76

CW, Day-old chick weight in gram; BW, body weights in gram at different weeks of age; ASM, age at sexual maturity (in days). Number of observation is 114 for each trait under each microsatellite. Means with different superscripts within a column under each microsatellite differ significantly (P≤0.05).

body weights under different genotypes of ADL0158 microsatellite locus were found statistically indifferent but in the trend of BE ≥ EE ≥ DD ≥ CE genotypes, though the former three genotypes had significantly higher estimates (>) than AD, BD and DE genotypes. Again, EE genotype had significantly higher body weight estimates at 16th and 20th week of age followed by AD ≥ DD ≥ BE > CE ≥ DE > BD for 16th week and BE > DD ≥ DE > AD ≥ CE > BD for 20th week estimates, thus our insight into the genotype to genotype comparison could demonstrate that the microsatellite genotypes EE (189:189 bp) and BE (219:189 bp) of ADL0158 locus would be the most beneficial in respect to body weight growth and could be exploited in molecular breeding. The least age at sexual maturity (ASM, 127.39±4.23 days) was observed in AB (160:147 bp) genotype followed by statistically indifferent BB (147:147 bp) genotype of ADL0273 locus and hence the most desirable by the breeders. Again, CC (267 bp allele) genotype of MCW0103 locus had the least ASM (132.46±2.46 days) among its other genotypes and was found better than BB genotype of ADL0273 locus. MCW0103 microsatellite genotypes AB (280:273 bp) and CC (267:267 bp) were statistically indifferent for body weight estimates at eighth and 16th week of age, and had significantly higher estimates than for BB, AA and BC genotypes. Again, AB heterozygote of MCW0103 locus had significantly higher estimate for BW20 than the other genotypes. Thus, MCW0103 locus demonstrated that its AB and CC genotypes uniformly favored body weight growth throughout the pre-housing age (16th week) while its CC favored lower ASM. AB (183:174 bp) genotype of MCW0069 locus had significantly the highest day-old chick weight estimate followed by AD ≥ CC ≥ BD ≥ AC ≥ BC genotypes. Similarly, CD (107:102 bp) genotype of MCW0110 locus performed the best (P≤0.05) in 16th week body weight estimate followed by BE ≥ EE ≥ CE ≥ BB ≥ AD ≥ AC ≥ BD ≥ CC ≥ DD ≥ BC genotypes. AE (216:147 bp) genotype of MCW0258 locus demonstrated the highest (P≤0.05) body weight estimate at eighth week of age followed by EE ≥ DD ≥ AD ≥ AC > BE genotypes. These findings were in the line of earlier reports of Das *et al.* (2016), Yadav *et al.* (2015) and Chatterjee *et al.* (2008, 2010). One-to-one correspondence, in the form of significance, between microsatellites and quantitative traits like chick weight, body weights and age at sexual maturity might be the informative indicator for elucidating microsatellite and QTL relationships (Sewalem 2002, Schreiweis 2005, Chatterjee *et al.* 2008, Chatterjee *et al.* 2010). The genetic principle of significant association of microsatellites and quantitative traits is possibly due to the phenomenon of linkage and if the microsatellite locus be very closely linked (about 20 cM) with a certain QTL, it will specifically be observed in terms of a significant association (Sewalem 2002, Schreiweis 2005, Chatterjee *et al.* 2010) which was observed in the present study, though the linkage analysis 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 quantitative traits in more accuracy. However, the present investigation might conclude that few microsatellites had influence on pre-housing body weights and age at sexual maturity of the present selected line of RIR chicken and the beneficial genotypes of these microsatellites could be favored in inheritance for molecular breeding program if conducted in this line for further genetic improvement which although thrives for further study with QTL analysis on larger sample size to reach more accurate conclusion.

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