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Study on the Correlation Between Microsatellite Markers and Body Weight in F_2 Populations of Chicken

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Abstract: The genomes of 500 individuals in F_2 population of Xianju chicken and Recessive White Chicken were screened using 26 microsatellite markers. The correlation between microsatellite markers and body weight were analyzed. The results showed that ADL 212 and LEI 147 on chromosome 2 were significantly associated with birth weight and weight at 12 week-age and the dominant genotype of these two loci were 118/103 and 295/262. MCW 4 and ADL 123 were significantly associated with birth weight and weight at 12 week-age, MCW 150 was related to 12 week-age weight. Meanwhile, MCW 223 and ADL166 on chromosome 5 were associated with birth weight, MCW 301, MCW 67 and ADL 210 on chromosome 8, 10 and 11 were associated with weight at 12 week-age.

Key words: Chicken, microsatellite, body weight

INTRODUCTION

In the last decade, Selection for broiler chickens has been focused on the improvement of the growth rate. As the living conditions improved, the needs not only for meat production but for meat quality are also increased especially in some developing countries. In the process of breeding for high quality chickens, the growth rate is still the focus of producer on the basis of good meat flavor. Marker-Assisted Selection (MAS) is a method of choice to choose growth traits of broilers due to its high accuracy, short select generation and low breeding cost. So searching for genetic markers associated with meat quality or Quantitative Trait Loci (QTL) controlling these traits are of great importance. Microsatellites have gained widespread use in phylogenetics, conservation genetics, genome mapping, assessing genetic diversity and structure in population studies and identifying individuals and parentage due to their abundance and random distribution over the genome, high polymorphism, codominant nature, high reproducibility and relative ease of scoring by the polymerase chain reaction (Weigend and Romanov, 2001; Chung *et al*., 2006). In the present study, the genomes of 500 individuals in F_2 population of Xianju chicken and Recessive White Chicken were screened using 26 microsatellite markers. The correlation between microsatellite markers and birth weight, body weight at 12 weeks were analyzed using the least square analysis, then searching appropriate microsatellite markers, which affect these traits. The results may provide evidence for QTL mapping and marker-assisted selection breeding in chicken.

MATERIALS AND METHODS

Chicken population: With F_2 design, Recessive White chicken and Xianju chicken were selected as parents to establish a resource population. A total of 500 individuals originating from this resource population were analyzed in this study. Birth weight and weight at the age of 12 weeks were measured.

DNA isolation: Per individual, 0.4 mL whole blood was collected from the ulnar vein with heparin as anticoagulant. Then, 4 mL of DNA lysate solution [2M urea, 100 mM Tris-HCl (pH 8.0), 1% SDS, 100 mM EDTA] was added and the mixture was stored at 4°C. DNA was isolated by using a phenol/chloroform based method (Sambrook and Russell, 2001).

Microsatellite genotyping: Twenty six microsatellite markers were selected according to the published genetic maps of three resource populations. The information of these 26 markers was listed in Table 1.

The 25 uL PCR volume included 50 ng of genomic DNA template, 1.0 uM of each primer, 200 uM of each dNTP, 1.5 mM $MgCl_2$ and 1 U Taq DNA polymerase. The amplification involved initial denaturation at 95°C (10 min),

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Table 1: The information of the 26 microsatellite markers

Markers	Chromosome	Total no. of alleles	Range of allele sizes (bp)
MCW 145	1	6	197~242
MCW 248	1	6	208~258
ADL 105	1	3	147~167
ADL 185	2	6	118~163
MCW 264	2	7	204~274
ADL 212	2	5	100~129
MCW 185	2	8	202~253
LEI 147	2	7	254~327
MCW 150	3	5	217~269
MCW 004	3	4	181~240
ADL1 36	4	6	140~180
ADL1 66	5	6	128~173
MCW 223	5	3	175~203
MCW 95	8	3	227~263
ADL 301	8	4	126~147
ABR 322	8	4	134~160
MCW 135	9	6	131~180
ADL 211	9	5	103~141
ADL 231	10	5	114~159
MCW 67	10	4	177~209
ADL 210	11	5	106~153
MCW 44	12	5	166~198
ADL 225	13	4	152~182
MCW 104	13	5	192~248
ADL 289	23	4	171~187
ADL 123	E47W24	5	105~144

followed by 30 cycles of denaturation at 95°C (1 min), primer annealing at temperature 48-66°C (1 min), extension at 72°C (1 min) and a final extension at 72°C (10 min). The obtained fragments were detected on 2.0% agarose gel.

The PCR products were subjected to 10% polyacrylamide gel in 1 × TBE buffer and electrophoresed at 200 voltages for 2 h. The DNA bands on the gel were viewed by silver staining. Allele-size scoring was performed with KDSZD 2.0 software.

Statistical analysis: Statistical analysis of associations between different genotypes and body weight was performed using the GLMM. The statistical model is:

$$y = \mu + j + s + r + e$$

where:

- y = Observed weight.
- μ = The least square value.
- j = The effect of genotype to weight.
- s = The effect of sex to weight.
- r = The effect of reciprocal cross to weight.
- e = The random error.

Type III sums of squares results showed that there was no significant interaction effect between genotypes, sex and reciprocal cross, so we didn't consider interaction effects in the model. The data were given in the mean±standard error format.

RESULTS

Correlation between microsatellite markers and traits: The least square analysis of 26 microsatellite markers and traits showed that four microsatellite markers had significant effects on birth weight and weight at 12 weeks (Table 2). The markers were ADL 212, MCW 4, MCW 104 and ADL 123, with the numbers of alleles of 5, 4, 5 and 5, respectively, which indicated these markers might relate to the QTL of traits.

The effect analysis of microsatellite and birth weight: On locus ADL212, 115/107 genotype individuals had significant higher birth weight than individuals with 107/110, 107/103, 112/103, 118/107, 118/112, 122/115, 291/181 genotypes ($p<0.05$) and the difference between 115/107 and 107/103 genotype individuals was 2.76 g, which was the largest. On locus MCW 4, individuals with 210/194 genotype had significant higher birth weight than individuals with 181/181, 206/181, 210/181 genotypes ($p<0.05$). On locus ADL123, the birth weight differences between 135/120 and 144/129, 114/105 and 120/110 genotype were significant ($p<0.01$) and the difference was 5.21 g between genotype 135/120 and 120/110, which was the highest. On locus MCW 104, the differences between genotype 210/210 and other genotypes was significant ($p<0.05$) and the birth weight of genotype 210/210 was higher than others (Table 3).

The effect analysis of microsatellite and weight at 12 weeks: On locus ADL212, the differences between genotype 118/112 and other genotypes except 118/103 and 122/115 were significant ($p<0.01$). The mean weight of individuals with 107/100 and 107/103 genotype was relatively lower. Individuals with 118/112 genotype had 470.99 g heavier weight than individuals with 107/103 genotype. On locus MCW 4, the genotype 210/194 was the dominant genotype, which has significant higher weight than other genotypes ($p<0.01$). 194/181 has the significant difference with 181/181, 206/181, 210/181, 220/ 194, 220/200 and 233/206 ($p<0.05$). ADLv123 was on the linkage group E47W24, 135/120 has significant difference with others ($p<0.01$) and the weight of it was heavier than that of 120/110, 415.53 g. The 192/192 and 214/192 of MCW 104 have significant difference with others ($p<0.01$) (Table 4).

Table 2: Results of variance analysis of the relation between microsatellite markers and traits

Marker	F-value	Marker	F-value	Marker	F-value
MCW 145	0.847	MCW95	0.198	MCW4	2.118**
MCW 248	0.746	ADL301	1.907	ADL136	0.938
ADL 150	1.710	ABR322	0.927	ADL166	0.757
ADL 185	0.970	MCW135	0.682	MCW223	1.267
MCW 264	0.908	ADL211	0.518	ADL225	0.876
ADL 212	1.695*	ADL231	0.920	MCW104	1.783*
MCW 185	1.521	MCW67	1.449	ADL289	0.894
LEI 147	1.328	ADL210	1.400	ADL123	2.995**
MCW 150	0.752	ADL44	1.103	———	

Note: *(p<0.05) and **(p<0.01)

Table 3: Multicomparison for birth weight in genotypes of microsatelite loci

Marker	Genotype	No.	Birth weight	Marker	Genotype	No.	Birth weight
MCW 4	181/181	10	36.22±1.29^{a}	ADL 212	107/100	16	36.13±0.81^{a}
	194/181	10	38.10±1.15^{ab}		107/103	31	35.95±1.03^{a}
	200/181	55	37.86±0.81^{ab}		112/100	20	38.38±0.73^{ab}
	206/181	171	36.52±0.25^{a}		112/103	210	37.03±0.23^{a}
	210/181	106	36.46±0.34^{a}		115/103	50	37.13±0.92^{ab}
	210/194	6	40.33±1.29^{b}		115/107	39	38.71±0.72^{b}
	220/194	12	36.54±1.14^{a}		118/103	26	38.36±0.88^{ab}
	220/200	22	38.78±0.82^{ab}		118/107	25	36.63±0.82^{a}
	233/206	36	37.80±0.62^{ab}		118/112	29	36.02±1.19^{a}
	240/210	59	38.05±0.51^{ab}		122/112	17	36.89±1.21^{a}
MCW 104	192/192	3	38.28±0.58^{a}		122/115	8	35.96±1.23^{a}
	210/192	54	37.14±0.27^{a}		129/118	16	36.18±0.83^{a}
	210/210	13	43.00±2.25^{b}	ADL 123	114/105	394	36.79±0.15^{a}
	214/192	6	36.59±0.35^{a}		120/110	10	35.33±1.25^{a}
	214/210	8	36.44±1.37^{a}		135/120	64	39.59±0.40^{b}
	225/192	5	37.38±1.13^{a}		144/129	11	40.54±1.14^{c}
	225/210	20	37.00±1.18^{a}		——	——	——
	230/210	5	36.82±0.99^{a}		——	——	——
	235/210	3	36.68±0.44^{a}		——	——	——
	239/210	3	37.02±0.65^{a}		——	——	——
	248/230	54	37.79±0.86^{a}		——	——	——

Note: The small letter in the table means significant difference (p<0.05), the capital letter in the table means badly significant difference (p<0.01)

Table 4: Multicomparison for 12-week body weight in genotypes of microsatelite loci

Marker	Genotype	No.	Weight at 12 weeks	Marker	Genotype	No.	Weight at 12 weeks
MCW 4	181/181	10	1389.67±108.60^{a}	ADL 212	107/100	16	1330.26±68.95^{a}
	194/181	10	1620.20±97.14^{b}		107/103	31	1312.78±88.23^{a}
	200/181	55	1528.67±68.13^{ab}		112/100	20	1385.19±62.01^{abc}
	206/181	171	1418.90±20.81^{a}		112/103	210	1440.20±19.20^{abc}
	210/181	106	1371.61±28.66^{a}		115/103	50	1410.37±78.79^{abc}
	210/194	6	1951.33±108.60^{C}		115/107	39	1482.60±61.83^{abc}
	220/194	12	1325.13±95.99^{a}		118/103	26	1579.54±74.95^{bcD}
	220/200	22	1409.76±69.35^{a}		118/107	25	1458.01±69.61^{abc}
	233/206	36	1448.44±51.99^{a}		118/112	29	1783.77±101.37^{D}
	240/210	59	1499.44±42.83^{ab}		122/112	17	1331.29±103.13^{abc}
MCW 104	192/192	3	1617.21±48.73^{c}		122/115	8	1601.92±104.93^{cD}
	210/192	54	1431.95±23.05^{ab}		129/118	16	1424.60±70.52^{abc}
	210/210	13	1645.00±190.62^{c}	ADL 123	114/105	394	1429.48±13.82^{a}
	214/192	6	1471.84±29.37^{b}		120/110	10	1289.60±108.85^{a}
	214/210	8	1296.28±116.01^{ab}		135/120	64	1705.13±34.72^{B}
	225/192	5	1440.00±95.31^{ab}		144/129	11	1421.21±99.80^{a}
	225/210	20	1264.04±99.88^{a}		——	——	——
	230/210	5	1271.29±83.65^{a}		——	——	——
	235/210	3	1469.20±37.39^{ab}		——	——	——
	239/210	3	1451.39±54.80^{ab}		——	——	——
	248/230	54	1405.45±72.79^{ab}		——	——	——

Note: As before

DISCUSSION

Microsatellite markers, which affected the growth traits were determined by GLMM analysis. They were ADL 212, MCW 4, MCW 104 and ADL 123 on Chromosome 2, 3 and linkage group E47W2 4, respectively. The least square analysis showed that the birth weights of genotype 115/107, 112/100 and 118/103 on marker ADL 212 were heavier and the difference between 115/107 and 107/103 was the largest with the value 2.76 g. The weights at 12 weeks of genotype 118/103, 122/115 and 118/112 were heavier. On this locus, the dominance genotypes of birth weight and weight at 12 weeks were different. So, when select the markers, focus should be on genotype 118/103 and 115/107. The difference between genotype 210/194 and 181/181, 206/181, 210/181 on marker MCW 4 is significant ($p<0.05$) and birth weight of 210/194 is higher than that of other genotypes, the highest value was 4.11 g. The birth weight and weight at 12 weeks of genotype 210/194 and 194/181 were higher than that of other genotypes and the difference was significant. Genotypes 135/120 and 144/129, 114/105 and 120/110 on marker ADL 123 had significant effects on birth weight ($p<0.01$). The difference between 135/120 and 120/110 was the largest. The difference of the effect on weight at 12 weeks between 135/120 and other genotypes was badly significant ($p<0.01$). Therefore, genotype 135/120 was the dominance genotype for birth weight and weight at 12 weeks. Marker MCW 104 was on Chromosome 5 and the difference between genotype 210/210 and others was significant ($p<0.05$). The birth weight and weight at 12 weeks of 210/210 were higher than that of other genotypes.

Sewalem *et al.* (2002) analyzed interval mapping QTLs in an F_2 chicken population established from a cross of a broiler sire-line and an egg laying (White Leghorn) line at 3, 6 and 9 wk body weights, using 101 microsatellite markers, the QTL significant at the genome wide level that affected body weight at 2 ages were identified on chromosomes 1, 2, 4, 7 and 8 and a QTL on Chromosome 13 influenced body weight at all 3 ages. van Kaam *et al.* (1998, 1999) and Tatsuda *et al.* (2000, 2001) found that QTLs on Chromosome 1 and 3 might influence the body weight.

Markers MCW 301, MCW 67 and ADL 210 on Chromosome 8, 10 and 11 had significant effect on weight at 12 weeks. Shu *et al.* (2007) showed that the marker ADL 212 on Chromosome 2 related with tenderness and pH value. The markers MCW 4 and MCW 223 on Chromosome 3 and 5 related with meat color and water lost rate. Bao *et al.* (2005) suggested the QTLs controlled the body weight may on the Chromosome 3 and linkage group E 27C 36W 25W 26.

CONCLUSION

Markers related with body weight of F_2 population the from the 26 microsatellite markers were mainly on Chromosome 2, 3, 5, 8, 10, 11 and linkage group E47W 24. These Chromosomes and linkage group might contain the QTLs, which controlled the body weight of F_2 population. The results may provide evidence for QTL mapping and MAS of chicken growth traits.

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