



Association of LEI-146 and MCW-43 microsatellites with body weights in bantam, bantamised White leghorn and White leghorn chicken

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ABSTRACT

Microsatellite markers have been studied for their association with growth traits in chicken. The object of this study was to investigate if the autosomal dwarf (*adw*) locus which is located in close proximity of LEI-146 and MCW -43 microsatellites, is associated with small body size of Bantam chicken. The samples were collected from 21 Bantam, 26 White Leghorn and 35 Bantamised White Leghorn birds and microsatellite analysis was carried out by 7% denaturing PAGE. Three and four alleles were found with LEI-146 and MCW-43 markers, respectively. The birds with having 1,1 and 1,3 genotypes for LEI-146 marker, had lowest body weight at all the ages, while birds with genotypes 2,2 and 3,3 were having highest body weight at all the ages. Birds with 1,3 and 4,4 genotypes for MCW43 markers were having highest body weight while birds with 1,2 and 2,3 genotypes were having lowest body weight at all the ages. Although the results point towards the association of these micro satellite loci with the body weight, they were statistically non-significant.

Contains 6 tables I figure and 2 plates

Key words: Microsatellite markers, bantam, body weight

Microsatellite markers are widely accepted as a marker of choice. They are highly polymorphic, single locus DNA sequences dispersed throughout the genome and are readily amenable to PCR. Microsatellites have been detected within genomes of every eukaryotes. Microsatellite markers are useful to study genetic variation, parentage assessment, gene flow, hybridization and genetic distancing (Bruford and Wayne, 1993; Cheng *et al.*, 1995 and Crooijmans *et al.*, 1996). ³²[P]-labelled synthetic oligonucleotide (GTG)₅ microsatellite probe was used to obtain DNA fingerprint patterns by hybridization with *Hinf I* and *Hae III* digests of individual DNAs from White, Leghorn and Kadaknath (Sharma *et al.*, 2000).

The White Leghorn (WLH) chicken has very high genetic potential to lay eggs in one year of laying cycle

while Bantam chicken has good feed efficiency with small body size. Their crosses are occasionally developed for improving the feed efficiency. It is essential to evaluate the genetic composition of such diverse genetic groups by characterizing those using genetic tools.

Microsatellite markers have also been studied for their association with some important economic traits like growth. Ruyster- Spira *et al.* (1998) have studied different microsatellite markers of LEI -146 and MCW -43 were closely linked with Autosomal Dwarf (*adw*) Locus in the chicken genome.

The present study was carried out with the object to investigate if the autosomal dwarf locus is associated with small body size of Bantam, through LEI-146 and MCW-43 microsatellite markers in Bantam, BWLH and WLH populations. The association of microsatellites and

body weights (BW) at different age has been studied here.

MATERIALS AND METHODS

Experimental birds: Experimental birds for the present study comprised of randomly selected 35 Bantamised White Leghorn (BWLH), 21 Bantam and 26 White Leghorn (WLH) birds from Central Poultry Research Station, College of Veterinary Science & Animal Husbandry, Gujarat Agricultural University, Anand. The BWLH stock was produced by reciprocally crossing WLH (IWN line) with Bantam subsequently backcrossing for two generations with WLH to have on an average 87.5 % inheritance from WLH.

DNA preparation: Four ml of venous blood was collected from wing vein of chicken in EDTA Vacuette (Greiner Labortechnik, Austria). The samples were transported on ice and subsequently stored at -4°C until processing.

DNA from the blood samples was isolated as described by John *et al.* (1997) with minor modifications. The quality and purity of DNA was carried out by UV - spectrophotometry and agarose gel electrophoresis.

Microsatellite analysis: PCR was carried out in IX PCR buffer with 1.5 mM MgCl₂, 200 μM dNTPs, 10 pmole each primers, 0.5 U Taq DNA polymerase, 100 ng template DNA in 25 ml reaction volume.

Two pairs of chicken microsatellite primers (Ruyster- Spira *et al.*, 1998) LEI-146 (FP: 5'-TCA AGC CAC CAAAGT GCT TGG -3' and RP: 5' -GAT CAC TCT GCT CAT AGC AGT -3') and MCW-43 (FP: 5' - TGA CT A CTT TGA T AC GCA TGG AGA3' and RP: 5'-CAC CAA GTA GAC GAA AAC ACA TTT -3') were used.

The cycling protocol followed for amplification was, initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 sec, annealing at 55 °C (54°C for MCW-43) for 45 sec, extension at 72 °C for 30 sec, final extension at 72 °C for 10 min.

Amplicons were confirmed by 2% agarose gel electrophoresis and the microsatellite alleles were resolved on non-denaturing 7% urea polyacrylamide gel.

Microsatellite bands were visualized by silver staining. **Statistical analysis:** Data on body weight at 8 weeks (BW8), 20 weeks (BW20), 40 weeks (BW40) and 56 weeks (BW56) were analyzed, breed and genotype wise, by two way analysis of variance using SPSS software. Heterozygosity, genetic distance were obtained with the help of PopGen analysis software (Yeh and Boyle, 1997). PIC values were calculated online at <http://jay.au.poznan.pl/-tomjan/ds.htm>.

RESULTS AND DISCUSSION

Autosomal dwarf locus is located on Chromosome 1 p at approximately 140 CM, while LEI146 and MCW-43 markers have been identified on the p arm of the same chromosome at 136 and 125 CM, respectively. The autosomal dwarf locus is closely associated with LEI - 146 marker at the distance of approximately 4 CM and that with MCW-43 is approximately 15 CM (Ruyster- Spira *et al.*, 1998).

Locus wise frequency: The locus wise frequency for both the primers is given in Table 1. Plates show the different genotypes identified on 7% non-denaturing urea PAGE by silver staining.

LEI-146 : The frequencies of different genotypes of LEI-146, varied in different breed groups, the variation was statistically non-significant. Total six genotypes (1,1; 1,2; 1,3; 2,2; 2,3; 3,3) were found with LEI-146. Genotypes 1,1 (0.238) and 3,3 (0.286) were at relatively high frequency in Bantam birds while 2,2 was absent in the Bantam population.

In case of BWLH birds, genotypes 1,3 (0.233) and 3,3 (0.300) were found at higher frequencies while 1,1 was having lowest frequency (0.033). WLH population was having highest frequency of 3,3 genotype (0.346) while genotype 2,2 (0.077) had lowest frequency.

Considering all the three populations, 3,3 genotype was having highest frequency (0.312) and 2,2 was having lowest frequency (0.065). However, the differences in genotypic frequencies were non-significant.

With the same microsatellite marker, a study was carried out on Kadaknath fowl (n=50) where the total 14

Table 1. Locus wise frequencies at LEI-146 and MCW-43 for different breed groups under study

Locus	Genotype	Bantam	BWLH	WLH	Overall	
LEI-146	1,1	0.238	0.033	0.115	0.117	
	1,2	0.190	0.133	0.115	0.143	
	1,3	0.143	0.233	0.115	0.169	
	2,2	0.000	0.100	0.077	0.065	
	2,3	0.143	0.200	0.231	0.195	
	3,3	0.286	0.300	0.346	0.312	
	Chi square		5.831 ^{NS}	1.453 ^{NS}	4.929 ^{NS}	7.314 ^{NS}
G square		6.755 ^{NS}	1.468 ^{NS}	4.682 ^{NS}	7.344 ^{NS}	
MCW-43	1,1	0.238	0.100	0.200	0.156	
	1,2	0.143	0.000	0.000	0.039	
	1,3	0.000	0.167	0.100	0.090	
	1,4	0.238	0.233	0.250	0.221	
	2,2	0.190	0.000	0.050	0.065	
	2,3	0.190	0.000	0.000	0.052	
	2,4	0.000	0.133	0.000	0.052	
	4,4	0.000	0.367	0.400	0.247	
	Chi square		15.636*	15.399*	45.124**	30.089**
	G square		18.051**	16.846**	15.855**	31.389**

genotypes were obtained which is comparatively higher than obtained in the present study (Rank, per com).

MCW-43 : The frequencies of different genotypes of MCW-43, varied in different breed groups, the variations were statistically significant. Total ten genotypes viz; 1,1; 1,2; 1,3; 1,4; 2,2; 2,3; 2,4; 3,3; 3,4; 4,4 were found in MCW-43 microsatellite marker. Genotype 1,1 (0.238) and 1,4 (0.238) were found to be with highest frequency in Bantam birds. Genotype 1,3; 2,4; 3,3; 3,4; 4,4 were absent in bantam birds.

BWLH birds were having highest frequency for genotype 4,4 (0.367) while 1,2; 2,2; 2,3; 3,3; 3,4 were absent in the BWLH population.

WLH was having highest frequency for genotype 4,4 (0.400) while 1,2; 2,3; 2,4; 3,3 were absent in WLH population. Pooled over three populations, genotype 4,4 was present with highest frequency (0.247).

With the same microsatellite marker, a study was carried out on Kadaknath fowl (n=50) where the total 15 genotypes were obtained which is

Table 2. Heterozygosity and PIC values for the markers in the breed groups under study

Locus	Bantam		BWLH		WLH		Overall	
	Hetero.	PIC	Hetero.	PIC	Hetero.	PIC	Hetero.	PIC
LEI-146	0.4762	0.545	0.5667	0.545	0.4615	0.546	0.5065	—
MCW-43	0.5714	0.60+	0.5333	0.533	0.3500	0.497	0.4930	—
Mean Std.	0.5238		0.5500		0.4058		0.4997	—
Dev.	0.0673		0.0236		0.0789		0.0096	—

comparatively higher than that obtained in the present study (Rank, per com).

Heterozygosity and polymorphic information content (PIC) : The results of Heterozygosity and PIC have been given in Table-2. Heterozygosity calculated based on LEI -146 and MCW -43 was found to be moderate. PIC was found similar in all the three populations for LEI -146 microsatellite marker.

Heterozygosity and PIC in Kadaknath fowl was found to be 0.569 and 0.434 for LEI -146 marker, respectively and 0.778 and 0.735 for MCW-43 marker, respectively (Rank, per com).

Table 3. Allelic frequency of LEI-146 and MCW-43 micro satellite markers in Bantam, BWLH and WLH population

Locus	Allele	Bantam	BWLH	WLH	Overall
LEI-146	1	0.4048	0.2167	0.2308	0.2727
	2	0.1667	0.2667	0.2500	0.2338
	3	0.4286	0.5167	0.5192	0.4935
MCW-43	1	0.4286	0.3000	0.3750	0.3592
	2	0.3571	0.0667	0.0500	0.1479
	3	0.0952	0.0833	0.0500	0.0775
	4	0.1190	0.5500	0.5250	0.4155

Allelic frequency: The allelic frequencies are presented in Table-3 for both the markers.

LEI-146: Total three alleles were found in LEI-146. Allele 2 was present in low frequency in Bantam birds (0.1667) while allele 1 (0.4048) and 3 (0.4286) had high frequency. Allele 3 was present in high frequency in BWLH (0.5167) and WLH (0.5192) population.

Total seven alleles were found in the same marker in the Kadaknath population. Allele 1 was found with high frequency (0.410) and Allele 3 with lowest frequency (0.020) in the population (Rank, per com).

MCW-43: Total four alleles were found in MCW43. Alleles 3 (0.0952) and 4 (0.1190) were present at low frequency in Bantam birds. BWLH (0.5500) and WLH (0.5250) birds were having high frequency of allele 4. Overall; allele 3 was in lowest frequency (0.0775) while

allele 1 (0.3592) and 4 (0.4155) were found in high frequency.

Total six alleles were found in the same marker in Kadaknath population. Allele 1 was found with high frequency (0.320) and Allele 3 with lowest frequency (0.040) in the population (Rank, per com).

Genetic identity and genetic distance: Statistics of genetic identity and genetic distance has been given in Table 4. The dendrogram shows the genetic distance between the breed groups (Figure-I). Bantam was genetically more distant than BWLH and WLH populations. BWLH and WLH populations were genetically more identical as WLH inheritance was more (87.5%) in the BWLH population.

Table 4. Measures of genetic identity and genetic distance for different breed groups under study

Populations	Bantam	BWLH	WLH
Bantam	-	0.7743	0.7975
BWLH	0.2558	-	0.9950
WLH	0.2262	0.0050	-

Note: Above diagonal is genetic identity and below diagonal is genetic distance

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Fig.1. Phylogenetic tree showing genetic distance between Bantam, Bantamised White Leghorn and White Leghorn

BWLH

WLH

The phylogenetic tree based on RFLP at growth hormone gene in cGH 1 and cGH2 motifs showed that WLH is at equidistance from BWLH and Bantam (Pipalia *et al.*, 2003). The phylogenetic tree based on different microsatellite data showed that BWLH and WLH were genetically close to each other, compared to Bantam and

Table 5. Genotype wise body weight in different breed groups for LEI-146 microsatellite

BW	Breed group		Genotypes					
			1,1	1,2	1,3	2,2	2,3	3,3
BW8	Bantam	Mean	245.60	236.00	240.00		268.66	253.33
		S.E.	24.73	27.65	31.93		31.93	22.57
	BWLH	Mean	294.00	387.00	391.57	334.66	355.66	364.22
		S.E.	55.30	27.65	20.90	31.93	22.67	18.43
	WLH	Mean	445.33	388.00	366.66	388.00	401.33	407.11
		S.E.	31.93	31.93	31.93	39.10	22.57	18.43
BW20	Bantam	Mean	647.20	606.00	618.66		642.66	621.33
		S.E.	41.37	46.25	53.40		53.40	37.76
	BWLH	Mean	1198.00	1260.00	1228.28	1246.66	1183.66	1295.77
		S.E.	92.50	46.25	34.96	53.4	37.76	30.83
	WLH	Mean	1272.66	1321.66	1236.66	1188.00	1312.00	1323.77
		S.E.	53.40	53.40	53.40	65.41	37.76	30.83
BW40	Bantam	Mean	774.60	729.25	813.33		872.66	707.83
		S.E.	73.95	82.67	95.47		95.47	67.50
	BWLH	Mean	1222.00	1398.50	1381.28	1380.66	1330.33	1462.66
		S.E.	165.35	82.67	62.50	95.47	67.50	55.12
	WLH	Mean	1526.66	1410.66	1430.00	1544.00	1436.66	1551.66
		S.E.	95.47	95.47	95.47	116.92	67.50	55.12
BW56	Bantam	Mean	731.00	715.50	799.33		829.33	686.00
		S.E.	77.58	86.73	100.15		100.15	70.82
	BWLH	Mean	1418.00	1581.50	1515.14	1502.66	1367.00	1657.11
		S.E.	173.47	86.73	65.56	100.15	70.82	57.82
	WLH	Mean	1605.33	1626.00	1461.33	1527.00	1580.00	1699.55
		S.E.	100.15	100.15	100.15	122.66	70.82	57.82

forming one cluster (Pipalia,2002).

Breed and genotype wise analysis of body weight at different ages: Differences were significant in Bantam, Bantamised White Leghorn and White Leghorn for body weights at 8, 20, 40 and 56 weeks.

LEI-146 genotype: The statistics for body weight analysis corresponding with the genotypes in LEI -146 is given in Table-5. Genotype 3,3 had highest body weight at 20, 40 and 56 weeks in Bantamised White Leghorn and White Leghorn. Whereas, genotype 2,3 had highest body weight at 8, 40 and 56 weeks in Bantam. However, the differences

were statistically non significant. No specific pattern was observed for other genotypes.

MCW-43 genotypes: The statistics for body weight analysis corresponding with the genotypes in MCW43 is given in Table - 6. Genotype 2,2 had highest body weight in Bantam at BW20, BW40 and BW56. This genotype was absent in Bantamised White Leghorn. Genotype 4,4 had highest body weight in Bantamised White Leghorn and White Leghorn at BW40 and BW56, however, differences were non significant. It is pertinent to note that 4,4 genotype was absent in Bantam. No specific pattern

Table 6. Genotype wise body weight in different breed groups for MCW -43 microsatellite

BW	Breed group		Genotypes							
			1,1	1,2	1,3	1,4	2,2	2,3	2,4	4,4
BW8	Bantam	Mean	224.00	256.66		252.00	254.00	263.00		
		SE.	24.18	31.21		24.18	27.034	27.034		
	BWLH	Mean	356.66		396.20	341.71			353.50	390.72
		SE.	31.21		24.18	20.43			27.03	16.30
	WLH	Mean	387.00		441.00	379.60	392.00			395.75
		SE.	27.03		38.23	24.18	54.06			19.11
BW20	Bantam	Mean	622.00	593.33		628.40	647.00	638.00		
		SE.	43.028	55.54		43.02	48.10	48.10		
	BWLH	Mean	1166.66		1280.80	1266.85			1238.00	1264.72
		SE.	55.54		43.02	36.36			48.10	29.00
	WLH	Mean	1285.25		1248.00	1261.60	1356.00			1316.00
		SE.	48.10		68.03	43.02	96.21			34.01
BW40	Bantam	Mean	785.20	705.33		757.60	817.50	748.75		
		SE.	71.89	92.80		71.89	80.37	80.37		
	BWLH	Mean	1232.00		1422.20	1365.71			1339.00	1462.54
		SE.	92.80		71.89	60.75			80.37	48.46
	WLH	Mean	1428.00		1496.00	1400.60	1400.70			1579.50
		SE.	80.37		113.66	71.89	160.75			56.83
BW56	Bantam	Mean	752.20	725.00		728.40	820.50	664.75		
		SE.	82.16	106.07		82.16	91.85	91.85		
	BWLH	Mean	1377.33		1542.40	1491.14			1454.75	1615.36
		SE.	106.07		82.16	69.43			91.85	55.39
	WLH	Mean	1584.00		1535.00	1534.80	1632.00			1707.25
		SE.	91.85		129.90	82.16	183.71			64.95

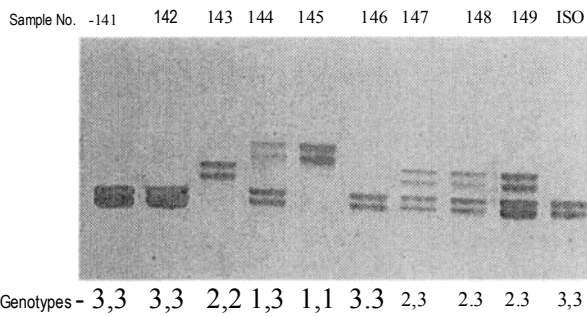


Plate I. Different genotypes of microsatellite marker LEI-146 resolved on denaturing PAG and visualized by silver staining

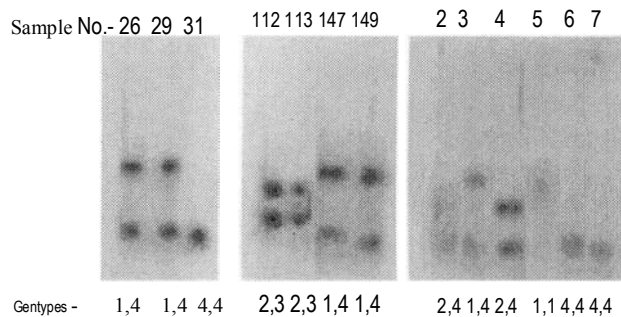


Plate 2. Different genotypes of microsatellite marker MCW-43 resolved on denaturing P AG and visualized by silver staining

was observed for *other* genotypes.

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