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RESEARCH ARTICLE

Microsatellite Markers Assisted Selection for High Body Weight in Local Broiler Breeders

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Abstract

The effect of selection for high 6-week live body weight (LBW) on the allelic frequencies of certain microsatellite loci in Cairo B-2 and RBC chicken lines were determined. The analysis covered 6 microsatellite markers within the two lines. Alleles were identified as genotype specific markers for Cairo B-2 and RBC lines. Selection for LBW appears to have affected the allelic frequency of tested microsatellite loci. The allelic number of the microsatellite markers MCW0010, MCW0018 and LEI0079, that are associated with live body, breast meat and carcass weight were higher in Cairo B-2 compared with the RBC line. The average heterozygosity in Cairo B-2 line was 0.063 and 0.109 for males and females respectively, while the RBC line shows higher values that reaches 0.375 for both males and females. Similar tendency was observed for the polymorphism information content (PIC) in which Cairo B-2 line show lower values (0.051 and 0.097) compared to the RBC line that showed higher values (0.269 and 0.297) for males and females respectively. The association between the important economic traits and microsatellite loci could facilitate the selection process by applying Marker Assisted Selection (MAS) in future breeding programs.

Introduction

Poultry geneticists face additional challenges today because of negative correlations between production and fitness traits. Accompanying selection for rapid growth, meat-type birds exhibit an increase in physiological disorders such as obesity, ascites, and leg problems, as well as a reduction in overall immune competence (Dunnington and Siegel, 1996 and Deeb and Lamont, 2002). Production performance and fitness traits are negatively correlated in chickens (Martin *et al*., 1990 and Pinard-van der Laan *et al*., 1998).

Meuwissen *et al*., (2001) reported that the traditional genetic selection, based on progeny testing, works very well but it is slow and expensive. Scientists posited that more accurate evaluations could be obtained by genomic selection, which uses genomic information from a large number of molecular markers. The recent growth of knowledge in molecular genetics, as applied to animal breeding, has opened new possibilities for improving broiler performance of specific broiler and breeder crosses (Toosi *et al*., 2010).

Currently, microsatellites (MS) are widely used since they are numerous, randomly distributed in the genome, highly polymorphic, and show co-dominant inheritance. The application of microsatellites became a standard technique for molecular genetics evaluation and mapping of chicken chromosomes (Cheng, 1997 and Gholizadeh and Mianji, 2007).

More than 800 highly polymorphic microsatellite markers are available in the chicken genome, which allows scanning for markers linked to economic traits of interest by using a genome wide search (Groenen *et al*., 2000).

The MS markers abundance, degree of polymorphism, ease of identification, and low mutation rates in different genomes make MS the preferred genetic markers for estimating genetic variation (Milligan *et al*., 1994 and Paszek *et al*., 1998). Compared to other DNA polymorphism assays, MS polymorphism detection results in the greatest expected heterozygosity (Powell *et al*., 1996).

Several MS markers are associated with chicken live body weight (LBW) at 6 weeks of age such as MCW0010 (Liu *et al*., 2007 and Zhang *et al*., 2008), MCW0018 (Sewalem *et al*., 2002 and Navarro *et al*., 2005), LEI0079 (Liu *et al*., 2008), ADL0251 (Van Kaam *et al*. 2002), c3-77696549 and c5-105790 (Uemoto *et al*., 2009) have been reported.

The objective of the current study was to determine the effects of eight generations of selection, for increased 6-week body weight, on the allelic frequency of microsatellite markers associated with live body weight, carcass parts and internal organs.

Materials and Methods

Experimental populations and management.

Selected males and females, from the eighth selected generation of Cairo B-2 line, were mated to produce the ninth generation (Nassar, 2008). Also, males and females from the eighth generation of the RBC line were mated to produce the RBC chicks. About two thousand Cairo B-2 and one thousand RBC pedigreed chicks' were wing banded and sexed at hatch, using the vent method. All chicks were reared intermingled, 10 birds/m^2, in an open house, deep litter system.

Birds were provided with a commercial broiler starter (23% CP and 3,050 kcal ME/kg) and a broiler grower (21% CP and 3,100 kcal ME/kg) diets from 1 to 14 days and from 15 days to 6 weeks of age respectively. Water and feed were provided *ad libitum* form hatch until 6 weeks of age. Light was provided 24 hours per day.

All baby chicks were vaccinated against avian influenza virus by using (S/C) injection of H5N2 inactivated vaccine at one week of age. Chicks were also vaccinated against Newcastle disease at 7 days (eye drop, Hitchner, Nobilis®), at 10 days (S/C injection with Newcastle inactivated vaccine, Nobilis®), and at 21 days (eye drop, La Sota strain, Nobilis®). Chicks were also vaccinated against infectious bursal disease at 14 and 24 days (eye drop) using Gumboro D78 strain (Nobilis®).

Blood samples and DNA isolation.

Individual genomic DNA was isolated from venous blood collected in anti-coagulate buffer from 15 males and 15 females from each line at 6 weeks of age. The extraction was carried out according to the method described by Bailes *et al*., (2007). Six microsatellites (MS) were chosen from a total of 10 MS that are associated with chicken live body weight and carcass parts and internal organs according to the public chicken genome database (http://www. ncbi.nlm.nih.gov/genbank/). The nucleotide sequences of the microsatellites used in this study are presented in Table (1).

The reaction mixture (20 µl) contained 50 ng DNA, 200 µM dNTPs, 1 µM from each primer, 0.5 unit of Red Hot *Taq* polymerase (AB-gene House-UK) and 10 X *Taq* polymerase buffer (AB-gene House-UK). The PCR conditions were as follows: 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 5 min. The PCR products were electrophoresed at 100 V on a 1.5% Agrose gel and visualized by staining with ethidum bromide.

Heterozygosity and polymorphism information content.

Heterozygosity (H) and polymorphism information content (PIC) per microsatellite marker were calculated according to Nagy *et al*., (2012).

Table 1.The used microsatellite primer codes and sequences and their distribution in chicken chromosomes.

Chicken Chromosome No.	Primer code	Sequence (5′ → 3′)	Expected fragment size (bp)	Ref.
1	MCW0010	F- TCTGTAGAATTACAGAAATACA R- TAGTACAAGAATCTAGTGTTAAAA	93-109	Croojimans *et al*., 1996
1	MCW0018	F- GGAATTTGAACACCTGAGATTTCC R- CACTATATGTTTATGGCAAACTCCTG	199-221	Croojimans *et al*., 1996
1	LEI0079	F- AGGCTCCTGAATGAATGCATC R- TCATTATCCTTGTGTGAAACTG	207–214	Liu *et al*., 2008
1	ADL0251	F- TTTGGCTTAGGGTGATGCTG R- CGTGCTCCACACAGGAATGT	200- 350	Van Kaam *et al*., 2002
3	c3-77696549	F- CAGGGAGTAGTTTGGTGCAA R- GCTTTCTTCAGTGCATTTTCG	278	Uemoto *et al*., 2009
5	c5-105790	F-GGAAGGGACTCCTACCTGCT R-AAATGGGTCTCTTGGTGCAG	167	Uemoto *et al*., 2009

Statistical analysis

Correlation coefficients between the allelic number and all the studied traits were obtained using the SAS software (SAS Institute, 2008).

Results and Discussion

DNA samples from the 9th selected generation of the Cairo B-2 and the RBC lines were subjected to PCR analysis using six specific (SSR) markers associated with LBW and carcass characteristics. The results indicated that, the allelic frequencies of the simple sequence repeats SSR loci MCW0010 were higher (eight alleles) in the Cairo B-2 line males and females, while the RBC lines shows only seven alleles (Table 2). A similar trend was also observed in which the selected males and females of Cairo B-2 line showed higher allelic frequencies for the MCW0018, LEI0079, ADL0251, c3-77696549 and c5-105790 compared to their corresponding males and females from the RBC line (Table 2).

There are higher allelic numbers, and consequently higher polymorphism, for all the MS markers tested in Cairo B-2 line, in comparison with the RBC line (Figs. 1 and 2). These results indicate that the genetic selection had an effect on the evolution of markers polymorphism within the genome of Cairo B-2 line which is associated with higher LBW at 6 weeks of age. These results are in agreement with the results previously reported by Ikeobi *et al*., (2004) and Loywyck *et al*., (2008). Also Ikeobi *et al*., (2004) and Nones *et al*., (2006) reported that, the MS marker MCW0010 is associated with breast muscles, leg muscles and carcass weights.

Table 2. Total allelic number for the microsatellite markers of the Cairo B-2 and RBC lines.

Marker name	Located Chromosome	Male		Female	
		Cairo B-2	RBC	Cairo B-2	RBC
MCW0010	1	8	7	8	7
MCW0018	1	6	3	7	6
LEI0079	1	8	7	7	5
ADL0251	1	8	4	7	4
c377696549	3	8	7	8	7
c5-105790	5	8	6	8	5
Average		**7.67**	**5.67**	**7.5**	**5.67**

The MS marker MCW0018 is associated with chicken carcass parts weights (Nones *et al*., 2006). Also, the MS marker MCW0018 is associated with chicken breast meat and abdominal fat weights (Lagarrigue *et al*., 2006). The high association between the weights of body parts and whole body weight could confound the identity of genes controlling variability in body weight with those involved in carcass composition variability (Lagarrigue *et al.*, 2006).

The results of the present investigation are also a in agreement with the results of Navarro *et al.*, (2005) in which LEI0079 and MCW0010 are associated with heart weight and the marker MCW0018 is associated with carcass parts and spleen and gizzard weights. Polymorphisms in the MCW0010 marker are suggestively associated with the QTL affecting the deposition of abdominal fat in broiler chickens (Liu *et al.*, 2007, Liu *et al.*, 2008 and Zhang *et al.*, 2008). Therefore selection to reduce abdominal fat may benefit from the implementation of marker-assisted selection (MAS).

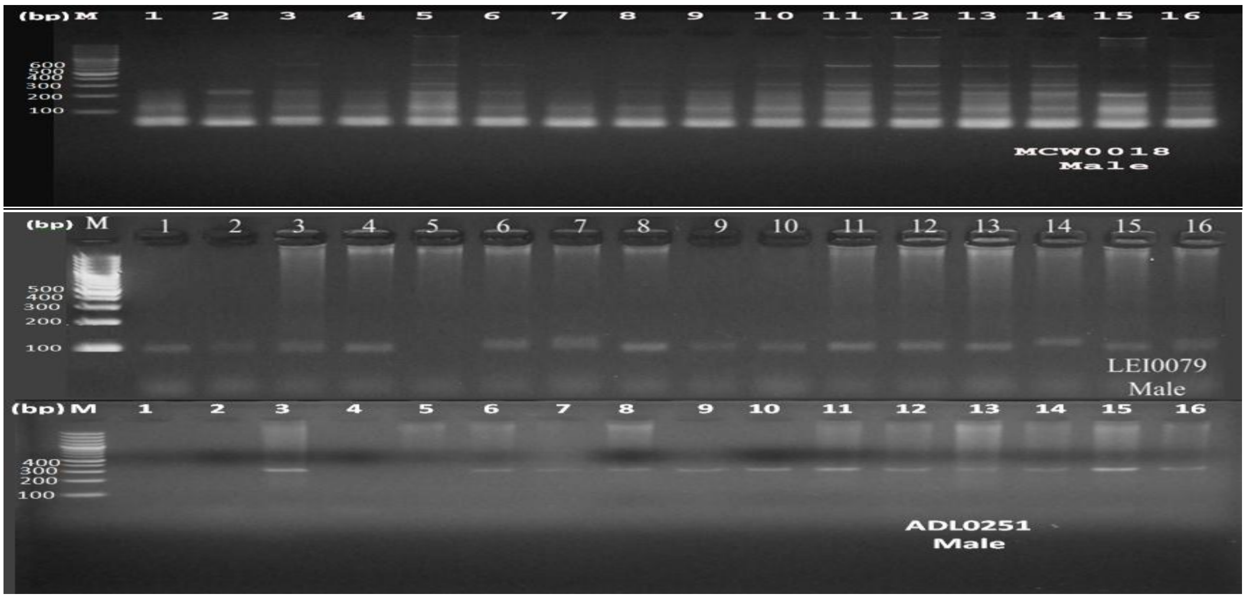

Fig. 1. Males PCR products and allelic numbers from both Cairo B-2 and RBC lines for the microsatellites used. Lanes: 1 to 8 are RBC line and lanes 9 to 16 are Cairo B-2 line. M, 100 bp DNA ladder (Fermentas Life Science, UK).

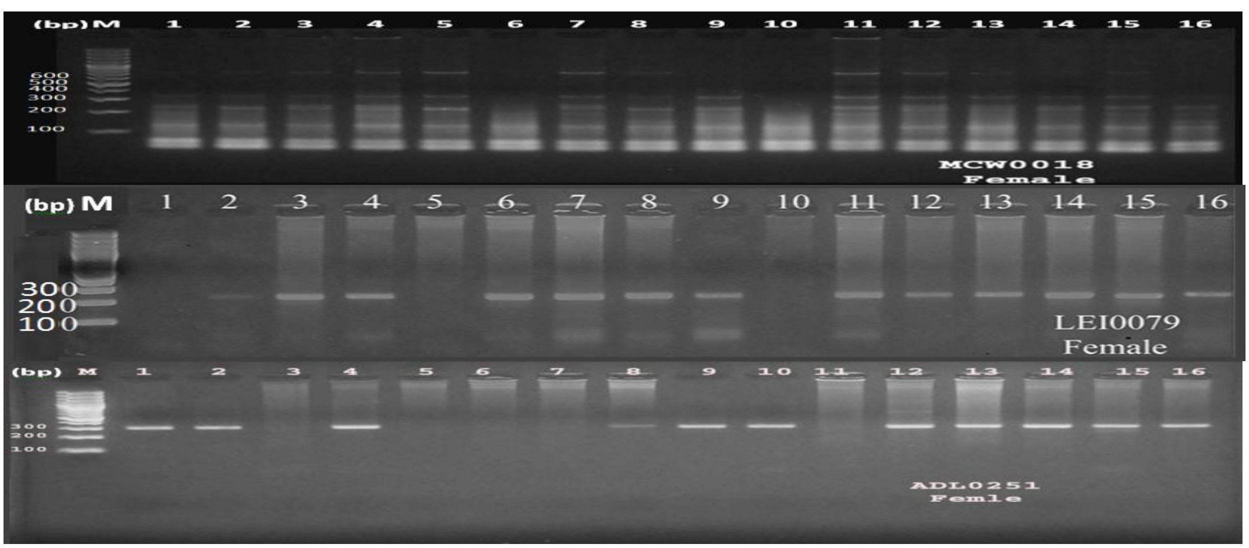

Fig.2. Females PCR products and allelic numbers from both Cairo B-2 and RBC lines for the microsatellites used. Lanes: 1 to 8 are RBC line and lanes 9 to 16 are Cairo B-2 line. M, 100 bp DNA ladder (Fermentas Life Science, UK).

Our results indicated that Cairo B-2 line had higher allelic number than the RBC line for the MS markers: MCW0018, MCW0010, LEI0079, ADL0251, c3-77696549 and c5-105790 (Table 2). The average number of alleles for the Cairo B-2 line males was 7.67, while it was 5.67 alleles for the RBC line (Table 2).The higher allelic numbers of the Cairo B-2 line indicates that selection for high 6-week body weight was associated with increasing the number of alleles for the chosen microsatellites. The high genetic associations between chicken LBW with carcass traits, indicates that direct selection for LBW at 6 weeks of age will result in indirect genetic gains for carcass, breast muscles, and leg muscles weights (Gaya *et al*., 2006 and Sandercock *et al*., 2009). Both Nones *et al.*, (2006) and Zhou *et al.*, (2006) reported an association between the MS marker ADL0251 and the live body weight at 6 weeks of age. This is in agreement with our results.

The previous finding are in agreement with Nassar *et al.*, (2012) who reported that the Cairo B-2 line had more allelic numbers than that of the RBC line for many microsatellite markers after six generation of selection for high LBW. The results also indicated that, the correlation coefficients between the allelic number of the different microsatellite markers tested and almost all studied traits, at 6 weeks of age, for both the Cairo B-2 and RBC lines were positive, and significant (Table 3).

Our results indicated that the correlations between the allelic numbers of the microsatellite marker MCW0010 with almost all the studied traits (Table 3) are positive and highly significant ($p<0.01$). The association between the allelic number of MCW0010 with heart weight was only significant ($p<0.05$).

The correlation coefficient between the allelic number of the marker MCW0018 and most studied traits (Table 3) are positive and significant ($p<0.05$). These results are in agreement with the results of Nassar *et al*., (2012) who reported positive and significant correlation coefficients between the allelic numbers of the microsatellite marker MCW0018 and almost all their studied traits.

On the other hand there are positive and highly significant correlations between the allelic number of the microsatellite marker LEI0079 and all studied traits (Table 3). The microsatellite marker ADL0251 showed positive and highly significant correlations with the live body weight, carcass weight, breast weight, wings weight and shank length. However, leg meat, abdominal fat, liver, heart, gizzard, giblets weights and keel length are correlated positively and significantly ($p<0.05$) with the allelic number of the microsatellite marker ADL0251.

Positive and highly significant correlations between the allelic number of the microsatellite marker c3-77696549 and most studied traits are present (Table 3). The correlation coefficients between the allelic number of the microsatellite marker c5-105790 and most studied traits are also positive and significant. However the correlation coefficient between the allelic numbers of the microsatellite marker c5-105790 and heart weight is not significant. These results agree with the results of Uemoto *et al*., (2009) who reported positive and significant correlation coefficients between the microsatellite markers c3-77696549 and c5-105790 and almost all their studied traits (body weight, carcass weight, barest meat and leg meat weights).

However, our results disagree with the results of Nassar *et al*., (2012), who reported no significant correlations between the allelic numbers of the microsatellite marker MCW0010 and all the traits that they studied, at 6 weeks of age, for both the Cairo B-2 and RBC lines.

Table (3). Correlation coefficients between the allelic number of the different microsatellite markers and all studied traits at 6 weeks of age for both the Cairo B-2 and RBC lines.

Traits / MM	LBW	Carcass wt.	Breast meat wt.	Leg meat wt.	Wings wt.	Abdominal fat wt.	Liver wt.	Heart wt.	Gizzard wt.	Giblets wt.	Keel length	Shank length
MCW0010	0.38**	0.41**	0.40**	0.45**	0.45**	0.43**	0.45**	0.33*	0.46**	0.45**	0.44**	0.41**
MCW0018	0.33*	0.33*	0.35**	0.31*	0.31*	0.35**	0.28*	0.18^{NS}	0.28*	0.27*	0.30*	0.23^{NS}
LEI0079	0.39**	0.43**	0.41**	0.40**	0.49**	0.43**	0.47**	0.42**	0.48**	0.48**	0.46**	0.47**
ADL0251	0.36**	0.36**	0.35**	0.32*	0.38**	0.32*	0.29*	0.26*	0.32*	0.31*	0.32*	0.37**
c3-77696549	0.32*	0.34*	0.34*	0.36**	0.39**	0.38**	0.40**	0.33*	0.38**	0.40**	0.37**	0.36**
c5-105790	0.32*	0.33*	0.33*	0.34*	0.30*	0.35**	0.29*	0.17^{NS}	0.32*	0.29*	0.37**	0.33*

** Significant at 1% level
* Significant at 5% level
^{NS} Not significant
N= 60 per Microsatellite

Based on the present data, it is recommended to use the microsatellite markers MCW0010 and LEI0079 in future selection program aiming to improve Cairo B-2 line. These two markers showed the highest significant correlation coefficients with body weight, carcass percentages and breast and leg meat weights.

The average heterozygosity of the Cairo B-2 line was 0.063 and 0.109 for the males and females respectively, while it was 0.375 in both males and females of the RBC line (Table 4). The heterozygosity of Cairo B-2 line for the MS markers MCW0010, c3-77696549 and c5-105790 were equal to zero for both males and females (Table 4). Also the heterozygosity of the males of Cairo B-2 line for the MS markers LEI0079 and ADL0251 were equal to zero (Table 4). These results are in agreement with Zhou and Lamont, (1999) who reported that observed heterozygosity ranged from 0.003 to 0.735 and 0.181 to 0.863, respectively.

Vanhala *et al*., (1998) studied the genetic variability and divergence of eight chicken lines using nine microsatellite markers. The chicken lines included three White Leghorn hybrids, three Finnish Landrace lines, a Rhode Island Red line, and a broiler hybrid line. All the microsatellite loci were found to be polymorphic. The number of alleles varied from 4 to 13 per locus and from 1 to 10 per line. Heterozygosities ranged from 0.00 to 0.91. The highest (0.67) and lowest (0.29) mean heterozygosity per line was observed in the broiler and White Leghorn lines respectively.

Table (4). Heterozygosity of six microsatellite markers for both males and females Cairo B-2 and RBC lines.

Marker name	Cairo B-2 line		RBC line	
	Males	Females	Males	Females
MCW0010	0	0	0.218	0.218
MCW0018	0.375	0.218	0.468	0.375
LEI0079	0	0.218	0.218	0.468
ADL0251	0	0.218	0.5	0.5
c3-77696549	0	0	0.218	0.218
c5-105790	0	0	0.375	0.468
Average	0.063	0.109	0.375	0.375

The average polymorphism information content (PIC) in the Cairo B-2 line was 0.051 and 0.097 for males and females respectively. However, the PIC observed in the RBC line was 0.269 and 0.297 for males and females respectively. The PIC of the Cairo B-2 line for the MS markers MCW0010, c3-77696549 and c5-105790 were equal to zero for both males and females (Table 5). Also the PIC of the male of Cairo B-2 line for the MS markers MCW0018 and ADL0251 were equal to zero (Table 5).

Table (5).Polymorphism information content (PIC) of six microsatellite markers for both males and females Cairo B-2 and RBC lines.

Marker name	Cairo B-2 line		RBC line	
	Males	Females	Males	Females
MCW0010	0	0	0.194	0.194
MCW0018	0	0. 194	0.358	0.304
LEI0079	0.304	0.194	0.194	0.358
ADL0251	0	0.194	0.375	0.375
c3-77696549	0	0	0.194	0.194
c5-105790	0	0	0.304	0.358
Average	0.051	0.097	0.269	0.297

These results are in agreement with Zhou and Lamont, (1999) who reported that the polymorphism information content ranged from 0.172 to 0.847 in populations of Chinese chickens. Also, Ya-Bo *et al*., (2006) reported that the average polymorphism information content (PIC) had values between 0.560 and 0.641.

Conclusion

Six microsatellite markers that had been reported to be association with LBW were used in the present study. All the primers used resulted in the production of scrabble bands. The average number of alleles for the Cairo B-2 line was 7.6, while it was 5.6 for the RBC line. Many allelic bands differed in their frequency between Cairo B-2 and the RBC lines. The Cairo B-2 line has more allelic frequency than the RBC line. This was shown to be associated with better LBW of the Cairo B-2 line at 6 weeks of age.

The genotype specific microsatellite markers can be used as useful markers to improve poultry production and they can be applied in the future breeding programs. The associations between four microsatellite markers in chromosome one, one microsatellite marker in chromosome 3 and one microsatellite marker on chromosome 5 with 6 weeks live bode weight, carcass weight, breast meat, leg meat, wings, abdominal fat, liver, heart, gizzard, giblets weights and keel and shanks length were observed in both of Cairo B-2 and RBC lines.

The statistical analysis of the data revealed significant positive correlations between the allelic number of the microsatellite markers and all the studied traits. While, the associations between the MCW0018, in chromosome one, were not-significant with both heart weight and shank length. Similarly, the correlation between the allelic number of the microsatellite marker c5-105790, in chromosome 5, with heart weight was not significant.

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