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Potential Association Between Microsatellite Markers on Chicken Chromosomes 6, 7 and 8 and Body Weight

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Abstract: Selective Genotyping (SG) is a strategy used to reduce the total number of markers and animals to be genotyped in order to detect QTL in a large designed population. The objective of this study was to detect potential associations between microsatellite markers on chromosomes 6, 7 and 8 (GGA6, GGA7 and GGA8) and Body Weight at 42 days (BW42), using an F_2 population developed by crossing a broiler (TT) and a layer (CC) lines. Chickens were separated by sex and BW42 of 2,063 F_2 offsprings was adjusted for the hatch effect. Animals that presented extreme phenotypes, i.e. the 4.5% lightest and heaviest from the normal distribution curve of phenotypes were selected within dam families, comprising 170 chickens. The means and standard deviations from the lightest and heaviest groups were 801.5±93.8 g and 1,328.5±127.8 g, respectively. Fourteen parents (TT males and CC females) and the selected 170 F_2 chickens were genotyped with 25 microsatellite markers, seven on GGA6, 11 on GGA7 and seven on GGA8. Statistical analyses used the chi-square ($p<0.25$) to test the null hypothesis (H_0) that assumed equal allele frequencies between lighter and heavier groups, indicating no association between marker and trait. On GGA6 no association was detected. On GGA7, four markers were potentially associated with BW42: *ADL0279* (p=0.1976), *ADL0109* (p=0.0946), *ADL0315* (p=0.2343) and *ADL0169* (p=0.0054). On GGA8, markers *MCW0351* (p=0.1580) and *ADL0154* (p=0.1741) were also associated. These associations indicate potential QTL regions, where QTL interval mapping studies should be conducted on these chromosomes to identify regions that control BW42.

Key words: Interval mapping, poultry, QTL, selective genotyping

INTRODUCTION

QTL are chromosomal regions associated to quantitative traits, which contain an unknown number of genes that control these traits. QTL can be detected when QTL alleles are in linkage disequilibrium with a molecular marker (Bovenhuis *et al*., 1997). Because chicken commercial populations are outbred, several factors may affect the detection of QTL: the number of markers used and their distribution on the chromosomes as well as their polymorphism level, population structure such as the number of families and of genotyped animals. Consequently, high number of markers and genotyped animals are required to increase the power for QTL detection, even though this may involve a considerable rise in research time and costs.

An alternative to this situation is the Selective Genotyping (SG) procedure. This approach does not require that all animals be genotyped for all markers and it allows a preliminary analysis indicating markers that are associated to QTL (Muranty *et al*., 1997). SG is used to associate markers to quantitative traits by genotyping only the animals present on the top and bottom extremes of the phenotypic distribution curve for a trait. Markers significantly associated to a trait in SG may, in a second step, be used together with flanking markers for QTL interval mapping (Darvasi and Soller, 1992). Consequently, SG can increase the power to detect QTL for a selected trait.

The objective of this study was to employ selective genotyping to detect potential associations between microsatellite markers on chromosomes 6, 7 and 8 and body weight at 42 days of age in an F_2 population developed by crossing broiler and layer chicken lines.

MATERIALS AND METHODS

Experimental population: An F_2 population was developed by crossing a chicken broiler (TT) and a layer (CC) lines. Seven TT males were mated to seven CC females to produce the F_1 TC population. Each F_1 male was mated to three unrelated F_1 females, originating 21 dam families. Each F_1 couple generated approximately

95 chicks in 17 hatches, totaling 2,063 F_2 chickens. For more details about this population see Rosário *et al*. (2009).

Animal selection for SG was conducted within families, in order to equally represent all families. After adjusting for sex and hatch effects, the proportion of animals selected within each family was the 4.5% lightest and 4.5% heaviest at 42 days of age. A total of 170 F_2 animals (belonging to 20 dam families, ranging from 6 to 10 F_2 per family, totaling 84 males and 86 females) were selected. The percentage of selected animals met the convenience of setting up two plates of 96 wells for genotyping as suggested by Ruy *et al*. (2005).

All F_2 chickens were recorded for body weight at 35, 42 and 41 days of age, weight gain at 35, 41 and 42 days of age, eviscerated carcass, breast, back, wings, legs, head, feet and liver weights. Body Weight at 42 days (BW42) was chosen for selective genotyping because it was highly correlated (r>0.80) with the other traits.

DNA isolation: Blood samples from parents and F_1 were extracted from the brachial vein and F_2 animals through bleeding at slaughter. Genomic DNA extraction from blood samples was based on the use of a guanidine-detergent lysing solution (DNAzol®, Invitrogen), which permitted selective precipitation of DNA from cell lysate with alcohol 95%. All samples were quantified in a spectrophotometer and standardized for 20ng μL^{-1}.

Marker selection and genotyping: Fluorescent primers employed in this study were either provided by Michigan State University, through the U.S. National Animal Genome Research Program (http://www.genome.iastate.edu), or synthesized from sequences available in the ArkDB (http://www.thearkdb.org). Each reaction that had a final volume of 25 µL contained 4.0ng μL^{-1} of genomic DNA, 4.0 mM of $MgCl_2$, 0.4 mM dNTP (A,T,C,G), 50 mM KCl, 10 mM Tris-HCl pH 8.5, 0.06 U μL^{-1} Taq DNA polymerase, 0.2pmol μL^{-1} of each primer (forward and reverse). The PCR program used was: 2 min at 94°C for initial denaturation and 30 cycles of 1 min at 94°C, 1 min at annealing temperature 45-65°C (according to primer-pair used) and 1 min at 72°C, followed by a final elongation step of 10 min at 72°C.

Twenty-five markers were used, seven on GGA6, 11 on GGA7 and seven on GGA8. Genotyping was carried out in the MegaBACE capillary sequencer (GE Health care) and fragment size analysis performed with the Genetic Profiler software (GE Health care). PCR reactions were carried out for each marker separately, but three to four markers were combined according to amplicon size and primer fluorescence for genotyping. Samples were precipitated and resuspended in 4.75µL loading solution (GE Health care) and 0.25µL internal standard ET-ROX 400 (GE Health care).

Table 1: Associations between microsatellite markers and body weight at 42 days by selective genotyping

Marker	GGA[1]	Position (cM)[2]	Probability
ROS0062	6	58.0	0.6907
MCW0250	6	59.0	0.9638
ROS0003	6	91.0	0.9266
ADL0377	6	99.0	0.7730
ADL0142	6	113.0	0.6714
LEI0064	7	0.0	0.4448
ABR326	7	30.0	0.4742
ADL0107	7	51.0	0.9725
ADL0279	7	92.0	0.1976*
ROS0019	7	101.0	0.3845
MCW0236	7	109.0	0.3813
ADL0109	7	117.0	0.0946*
ADL0315	7	140.0	0.2343*
ADL0169	7	165.0	0.0054*
ABR0322	8	5.0	0.7919
MCW0095	8	26.0	0.7608
ADL0154	8	46.0	0.1740*
ABR0345	8	56.0	0.3393
ADL0301	8	80.0	0.6370
ADL0172	8	92.0	0.3992
MCW351	8	105.0	0.1582*

[1]*Gallus gallus'* chromosome, [2]position given by the chicken consensus linkage map (Schmid *et al*., 2005), *p<0.25

The chicken consensus linkage map (Schmid *et al*., 2005) was used to determine marker position in order to facilitate comparisons with results from the literature.

Statistical analysis: Phenotypic correlations based on the Pearson's coefficient between all pairs of traits were obtained using PROC CORR (SAS, 2001) with p<0.05 (data not shown). PROC FREQ of SAS (2001) was used to implement the analysis of SG, where allele marker frequencies of extreme phenotypes (tails of a normal distribution curve) were compared using chi-square tests (p<0.25). This p-value was adopted because SG is an exploratory analysis that we used to detect potential microsatellite markers, which define putative regions where QTL may be located. A more conservative significance level could result in loss of putative QTL regions when more sophisticated analyses are used, for example, interval mapping (Lander and Botstein, 1989). The null hypothesis (H_0) assumed equal allele frequencies among groups (light and heavy), i.e., the absence of association between marker and trait. The alternative hypothesis indicated significant potential association between marker and trait.

RESULTS AND DISCUSSION

We did not map QTL associated to BW42 here, but we aimed at detecting potential associations between microsatellite markers and BW42 in a Brazilian F_2 chicken population designed to map QTL for performance and carcass traits. Nones *et al*. (2006), using the same population that we used here, mapped QTL for performance and carcass traits on GGA1 by interval mapping.

Out of 25 markers tested in the present study, two from GGA6 (*LEI0192* e *ADL0040*) and two from GGA7 (*MCW0178* e *ADL0180*) showed only one allele in the F_2 animals, i.e. these markers were uninformative and were discarded from SG (*LEI0192* and *ADL0040* on GGA6, *MCW0178* and *ADL0180* on GGA7). Therefore, the average distances between markers was 11.0, 22.0 and 14.3 cM on GGA6, GGA7 and GGA8, respectively (Table 1).

SG did not indicate any association (p>0.25) between markers on GGA6 and BW42 (Table 1). On GGA7, four markers were associated to differences in BW42: *ADL0279*, *ADL0109*, *ADL0315* and *ADL0169*, whose type I error probability in chi-square test were p = 0.1976, p=0.0946, p=0.2343 and p=0.0054, respectively. Marker *ADL0169* at 165 cM showed the greatest probability of association with BW42 (Table 1). On GGA8, two markers were associated to BW42: ADL0154 (p=0.1741) and *MCW0351* (p=0.1580) (Table 1).

SG can significantly increase the power to detect QTL for a specific trait (Darvasi and Soller, 1992; Muranty *et al*., 1997; Bovenhuis and Spelman, 2000). However, the benefit of genotyping reduction decreases when the number of traits increases, mainly if correlations between them are low. When traits are not correlated, the extreme phenotypes of one trait and another could be different, causing a reduction in the statistic power to detect QTL. The power to detect QTL is only slightly reduced if the traits are highly correlated. In this study, we chose BW42 for SG because this trait showed high phenotypic correlation (r>0.80) with other recorded traits. According to Muranty *et al*. (1997), in non endogamic population crosses, if the selection of animals to be genotyped is done in the extremes of the phenotypic distribution curve from the whole population, there is a risk of selecting animals from a subset of families, due to family effect. Therefore, in the present study, animals were selected within F_1 dam families (20 families), consisting of two groups (lighter and heavier), whose means and standard deviations were 801.5±93.8 g and 1,328.5±127.8 g for BW42 in the lighter and heavier groups, respectively.

Although we did not find associations on GGA6, some studies have reported QTL close to the markers used in our study. Sewalen *et al*. (2002), using a cross between a broiler male line and a layer (White Leghorn) female line, mapped suggestive QTL on GGA6 for body weight at six and nine weeks between 58 and 91 cM on chicken consensus linkage map next to *ROS0003* and *ROS0062* markers. In an F_2 population originated by crossing two divergently selected lines for high (H line) or low (L line) primary antibody responses to sheep red blood cells, Siwek *et al*. (2004) mapped a suggestive QTL for body weight at six weeks between markers *MCW0325* and *ROS0028* (positioned at 59 and 100 cM, respectively). McElroy *et al*. (2006) mapped a suggestive QTL for body weight at six weeks at 88 cM, using a reciprocal cross of a broiler male line and a broiler female line. Markers used by Siwek *et al*. (2004), mentioned above, were not used in the present study, however, markers *MCW0250* and *ADL0377* are positioned on chicken consensus linkage map at 59 and 100 cM, respectively, coinciding or being very close to the positions of markers presented by those authors. Differences may be due to genetic background since broiler and layer lines were used to create the population used in this study as observed by Rosário *et al*. (2009).

On GGA7, QTL for body weight have been mapped between 0 to 100 cM (Abasht *et al*., 2006). Our results confirmed and expanded these observations. In a QTL mapping study for body weight at three, six and nine weeks, Sewalen *et al*. (2002), mapped 1% genome-wide significant QTL for body weight at three weeks, 5% genome-wide significant QTL for body weight at six weeks and a suggestive QTL for body weight at nine weeks, at 57 cM on GGA7. Our results are in agreement with those from Siwek *et al*. (2004), who mapped suggestive QTL for body weight at four and six weeks, between markers *LEI0064* and *MCW0236* (0-109 cM). Additionally, these authors also mapped QTL for body weight at eight weeks between markers *ABR0326* and *MCW0183* (30-86 cM).

Additionally, on GGA7, Jacobsson *et al*. (2005) using a third-generation pedigree by intercrossing two lines of White Plymouth Rock chickens divergently selected for juvenile body weight mapped a 5% genome-wide significant QTL for body weight at 42 days of age and a 1% genome-wide significant QTL for body weight at 56 and 70 days of age. These authors also mapped a suggestive QTL for growth from 56-70 days of age and a 5% genome-wide significant QTL for growth from 28-42 days and 42-56 days of age on the same chromosome. The QTL mapped were positioned between 50 and 80 cM, between markers *ADL0169* and *MCW0120*. Our results were slightly different from those authors, because the highest association was found at 165 cM (*ADL0169*). Thus, the present study suggests a new potential region that might contain QTL associated to BW42 or another correlated performance trait. Other studies must be carried out using more markers in this region to confirm this putative QTL.

Our results on GGA8 are in agreement with studies that have mapped QTL close to markers *ADL0154* (46 cM) and *MCW351* (105 cM). Sewalen *et al*. (2002) mapped a suggestive QTL on GGA8 for body weight at three weeks and a 5% genome-wide significant QTL for body weight at six and also at nine weeks, positioned at 95, 93 and 59 cM, respectively. Kerje *et al*. (2003) using a large intercross between the domestic White Leghorn chicken and the wild ancestor, the Red Junglefowl, mapped a 5% genome-wide significant QTL for body weight at eight days of age, next to markers *ADL0154* (46 cM), as observed in the present study.

Several authors reported QTL regions close to markers used here. However, the results from those studies were based on interval mapping analyses using a greater number of animals. SG strategy was employed in the present study as a fast and less expensive strategy to identify potential markers associated to body weight, thus providing an indication of putative QTL regions for further studies. Three and two regions associated to BW42 were detected on GGA7 and GGA8, respectively. Other microsatellite markers may be selected on these regions, allowing high saturation and probably more power to detect QTL. Finally, we will employ interval mapping using a larger number of animals and markers to map QTL for performance and carcass traits, defining respective positions and effects on the GGA7 and GGA8.

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