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Fine Mapping of QTL for Carcass and Meat Quality Traits in a Chicken Slow-Growing Line

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ABSTRACT: A QTL detection was performed on birds originated from a slow-growing line selected by the SASSO breeding company since 1994. More precisely, 764 chicken and their parents (10 sires and 87 dams) were genotyped on the Illumina chicken SNP 60K Beadchip. Measures of body weight, breast meat yield, abdominal fat yield, leg yield, pH at 15 min and 24h post-slaughter, meat color, drip loss and intramuscular fat content were registered. Two methodologies of fine mapping were tested: a linkage disequilibrium (LD) analysis (EMMAX) and a linkage disequilibrium and linkage analysis (LDLA). With the two methodologies, we detected a significant QTL of early growth at a p-value of $5x10^{-5}$ on chromosome 24. We detected also significant QTL of breast meat yield on the chromosomes 17 and 18 and a QTL of pH at 15 min post-mortem on the chromosome 13.

Keywords: chicken, slow-growing line, QTL, carcass quality, meat quality

Introduction

Chicken slow-growing lines were developed in the sixties for the high-quality Label Rouge production. These lines are slaughtered at a more mature age (a minimum of 81 days) than conventional fast-growing lines (slaughtered between 35 and 42 days). It has been shown that the slow-growing lines present a higher flavour intensity (Yamashita et al. (1976)) of the meat than the fast-growing lines. Because of the better meat quality and the rearing conditions of the Label Rouge chicken (low density, free access to outdoors), this production represents now a large part of the whole carcass market (56% in 2011). However, the market share of Label Rouge chicken for cuts and processed products is still limited (11% in 2011). To adapt this production to the cuts market, a better knowledge of the genetic determinism of growth, body composition and breast meat quality traits is needed. The objective of the study is to identify QTL of carcass and meat quality in a population of slow-growing chicken.

Materials and Methods

Data. The chicken originated from a slow-growing line selected by the SASSO breeding company (Sabres, France) since 1994. The experimental design and performances were described in details in a previous study (Chabault et al. (2012)). Briefly, birds were naked-neck chicken with yellow shanks. The pedigree was constituted of ten sires each mated with 10 dams for a total of 1022 male and female offsprings. Birds were reared in three successive batches (mixed sex) under similar free-range conditions. At 3, 6 and 9 weeks of age, birds were individually weighed (BW_3, BW_6, BW_9) and then at 12 weeks of age, birds were weighed (BW_12) and slaughtered after 7 hours' feed withdrawal. At 15 min post-mortem, the pH (pH15) was measured in the right Pectoralis major muscle. Samples of this muscle were frozen for further analyses of lipids composition. Carcasses were dissected 24h post-mortem. Breast meat yield (Pectoralis major plus minor, Breast Meat Y.), abdominal fat yield (Abd. Fat Y.) and leg yield (Leg Y.) were calculated as percentages of body weight at slaughter. All measurements of meat quality were then performed on the left pectoral major muscle. At 24h post-mortem, the pH (pHu) was measured. Colour (L*) was measured on the upper ventral side of the muscle. The drip loss was evaluated as the difference in weight of the breast Pectoralis major muscle before and after placed at 2°C for 5 days. After thawing for 12h at 4°C, intramuscular fat content (IMF) was determined on the previously frozen samples of the Pectoralis major muscle.

Genomic DNAs were extracted from blood samples of 764 chicken and their parents (10 sires and 87 dams). Genotyping was performed by the Labogena Laboratory (Jouy en Josas, France) using the Illumina chicken SNP 60K Beadchip (containing 57636 SNP).

After quality control, 25 samples were removed from the further analyses: 12 samples with a call rate < 95%, 2 samples with chimeric DNA and 11 samples due to incompatible genotypes with the parents. A total of 16340 markers (28%) were excluded for failing to meet one or more of the following conditions: SNP on autosomal chromosomes, SNP call rate > 95%, minor allele frequency (MAF) > 5%, Hardy-Weinberg equilibrium (HWE) test with a p-value > 10^{-4}. After these quality control steps, 836 birds (739 descendants, 10 sires, 87 dams) and 41296 SNP remained.

Model. Two methodologies were used to perform the QTL detection. The first methodology, EMMAX (Kang et al. (2010)) is based on a linear mixed model approach using a genetic relationship matrix estimated by high-

density SNP genotypes to model the sample structure. The contribution of the sample structure to the phenotype is estimated using a variance component model resulting in an estimated covariance matrix of phenotypes. Then a generalized least square (GLS) test is applied at each marker to detect associations.

The second methodology is a linkage disequilibrium and linkage analysis on haplotypes (4 SNP) estimating a matrix of IBD probabilities. This approach is based on the methodologies described by Meuwissen and Goddard (2000, 2001) and performed in a program of Druet et al. (2008). The genotypes were previously phased with programs of the PHASEBOOK (Druet and Georges (2010).

For the two analyses, we considered two thresholds of significance for the p-values of the tests: $5x10^{-4}$ and $5x10^{-5}$. In the LDLA analyses, the p-values were estimated considering that the distribution of the statistical test (The Likelihood Ratio Test LRT) is a mixture with equal weights $(\frac{1}{2}\chi_0^2 + \frac{1}{2}\chi_1^2)$ of a Dirac distribution (probability mass of one) at zero degree of freedom and of the usual chi-square distribution with one degree of freedom. The p-values of $5x10^{-4}$ and $5x10^{-5}$ correponds to a LRT of 10.8 and 15.1 respectively.

Results and Discussion

We detected 384 and 1656 QTL at the threshold of p-value=$5x10^{-4}$ with the EMMAX and LDLA methodologies respectively (Table 1). This high difference in the number of QTL detected suggests that the EMMAX methodology is very conservative or a high number of false-positive QTL detected by LDLA. At the threshold of p=$5x10^{-5}$, we found 122 and 703 QTL with EMMAX and LDLA respectively (Table 1).

Table 1: Numbers of QTL detected with a p-value < $5x10^{-5}$ in bold and a p-value <$5x10^{-4}$ in brackets, for the two methodologies.

Traits	EMMAX	LDLA
BW_3	**51** (91)	**232** (349)
BW_6	**22** (51)	**147** (261)
BW_9	**7** (33)	**76** (166)
BW_12	**2** (29)	**42** (184)
Breast Meat Y.	**22** (52)	**162** (290)
Abdominal Fat Y.	**1** (23)	**20** (148)
Leg Y.	**0** (18)	**3** (29)
pH15	**4** (22)	**2** (66)
pHu	**9** (26)	**1** (25)
L.	**1** (11)	**2** (30)
drip loss	**3** (21)	**1** (16)
IMF	**0** (7)	**15** (92)

Most of the QTL detected are QTL of body weight and breast meat yield.

In the Table 2, we reported the most significant position for each trait per chromosome at the threshold of p=$5x10^{-5}$. We found 9 common QTL between the 21 and 18 most significant QTL detected by EMMAX and LDLA respectively.

Table 2: The highest significant position for each trait per chromosome with a minimum p-value of $5x10^{-5}$. We indicate in bold the QTL detected by the two methodologies in an interval of 4 Mb.

Method	Trait	Chr.	Position (bases)
LDLA	Breast Meat Y.	1	24531335
LDLA	BW_9	1	159434732
EMMAX	**BW_12**	**1**	**166273503**
LDLA	**BW_12**	**1**	**169700738**
EMMAX	Abd. Fat Y.	3	111494269
EMMAX	pH15	4	30472266
LDLA	IMF	4	80111703
LDLA	IMF	5	8069203
EMMAX	drip loss	5	35024640
LDLA	**Breast Meat Y.**	**6**	**21040066**
EMMAX	**Breast Meat Y.**	**6**	**23528749**
EMMAX	pH15	7	16852999
LDLA	IMF	7	28069691
EMMAX	drip loss	9	16020519
EMMAX	pHu	10	10372217
EMMAX	BW_6	11	5530397
LDLA	BW_3	11	8242619
LDLA	BW_6	11	9932042
EMMAX	**BW_6**	**12**	**5246728**
LDLA	**BW_6**	**12**	**5542475**
LDLA	Leg Y.	12	5871954
LDLA	**pH15**	**13**	**9632557**
EMMAX	**pH15**	**13**	**9703415**
EMMAX	BW_3	14	5839570
EMMAX	pHu	15	5439436
EMMAX	L.	15	8345486
LDLA	**Breast Meat Y.**	**17**	**1873351**
EMMAX	**Breast Meat Y.**	**17**	**1937604**
LDLA	**Breast Meat Y.**	**18**	**513453**
EMMAX	**Breast Meat Y.**	**18**	**793653**
EMMAX	**BW_3**	**24**	**79759**
LDLA	**BW_3**	**24**	**461628**
LDLA	**BW_6**	**24**	**461628**
EMMAX	**BW_9**	**24**	**731393**
EMMAX	**BW_6**	**24**	**731393**
LDLA	**BW_9**	**24**	**792681**
EMMAX	pHu	24	1962830

LDLA	Abd. Fat Y.	26	3114552
EMMAX	BW_12	27	2933140

Several QTL of body weights were detected on the chromosome 24. The figures 1 and 2 show in details the QTL of body weight at 3, 6 and 9 weeks detected by the two methodologies. The significance of the QTL decrease with the age of the chicken, suggesting the presence of a QTL of early growth.

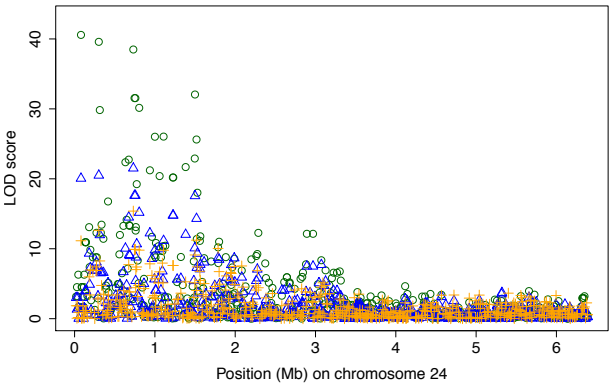

Figure 1: Distribution of the LOD score for the body weights at 3 weeks (o), 6 weeks (Δ) and 9 weeks (+) on the chromosome 24 with EMMAX.

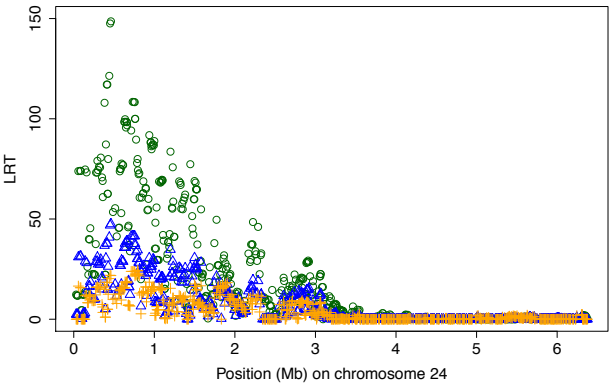

Figure 2: Distribution of the LRT for the body weights at 3 weeks (o), 6 weeks (Δ) and 9 weeks (+) on the chromosome 24 with LDLA.

Conclusion

At the threshold of $p=5x10^{-5}$, we detected 122 and 703 QTL of carcass and meat quality traits in the population of slow-growing chicken with EMMAX and LDLA respectively.

Among the most significant QTL detected by the two methodologies, we detected 9 common QTL.

To go further, we would like to study more precisely the QTL of body weight on the chromosome 24, the QTL of breast meat yield on the chromosomes 17 and 18 and the QTL of pH15 on the chromosome 13. We plan to sequence individuals with extreme phenotypes to identify causative candidate mutations.

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