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Original article

Confirmation of quantitative trait loci affecting fatness in chickens

Danyel G.J. JENNEN[a,b*], Addie L.J. VEREIJKEN[c], Henk BOVENHUIS[a], Richard M.P.A. CROOIJMANS[a], Jan J. van der POEL[a], Martien A.M. GROENEN[a]

[a] Animal Breeding and Genetics Group, Wageningen Institute of Animal Sciences, Wageningen University, Marijkeweg 40, 6709 PG Wageningen, The Netherlands
[b] Institute of Animal Breeding Science, University Bonn, Endenicher Allee 15, 53113 Bonn, Germany
[c] Nutreco, Breeding Research Centre, P.O. Box 220, 5830 AE Boxmeer, The Netherlands

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Abstract – In this report we describe the analysis of an advanced intercross line (AIL) to confirm the quantitative trait locus (QTL) regions found for fatness traits in a previous study. QTL analysis was performed on chromosomes 1, 3, 4, 15, 18, and 27. The AIL was created by random intercrossing in each generation from generation 2 (G_2) onwards until generation 9 (G_9) was reached. QTL for abdominal fat weight (AFW) and/or percentage abdominal fat (AF%) on chromosomes 1, 3 and 27 were confirmed in the G_9 population. In addition, evidence for QTL for body weight at the age of 5 (BW5) and 7 (BW7) weeks and for the percentage of intramuscular fat (IF%) were found on chromosomes 1, 3, 15, and 27. Significant evidence for QTL was detected on chromosome 1 for BW5 and BW7. Suggestive evidence was found on chromosome 1 for AFW, AF% and IF%, on chromosome 15 for BW5, and on chromosome 27 for AF% and IF%. Furthermore, evidence on the chromosome-wise level was found on chromosome 3 for AFW, AF%, and BW7 and on chromosome 27 for BW5. For chromosomes 4 and 18, test statistics did not exceed the significance threshold.

quantitative trait loci / advanced intercross line / chicken / fatness traits

1. INTRODUCTION

Fat deposition is an important trait in the chicken, which has been examined in several studies for the identification and localization of quantitative trait loci (QTL) (*e.g.* Tatsuda and Fujinaka [23]; Ikeobi *et al.* [14]; McElroy *et al.* [19]; Pitel *et al.* [20]). We previously identified QTL affecting fatness in a cross

* Corresponding author: djen@itz.uni-bonn.de

between two genetically different outcross broiler dam lines originating from the White Plymouth Rock breed [16]. This resulted in a genomewise significant QTL for the percentage of abdominal fat at the age of 10 weeks on chicken chromosome 1. This QTL explained about 18% of the total genetic variance. Furthermore, suggestive QTL for fatness traits were found on chromosomes 1, 2, 3, 4, 7, 11, 13, 15, 18 and 27. Confirmation of these QTL is an essential step before attempts are made towards the fine mapping of the QTL and the identification of genes underlying the traits of interest.

Confirmation of the presence and location of the QTL of interest can be achieved by comparing the results from different QTL studies. In the comparison of two granddaughter designs Bennewitz *et al.* [2] confirmed QTL affecting milk yield in cattle. In the study of two distinct layer × layer crossess, Siwek *et al.* [22] validated the presence of a QTL for the primary antibody response to keyhole lympet hemocyanin on chromosome 14 in both populations. Confirmation of QTL within a commercial broiler line was achieved by De Koning *et al.* [8]. In this study QTL for body weight and residual feed intake on chicken chromosome 4 were confirmed from results from other QTL studies reported in the literature. Some, but not all of the fatness QTL found in our previous study [16] were in the same chromosomal region as found by others [14,19,23]. The QTL regions are quite large (50−100 cM) and only partially overlapping. Furthermore, different phenotypic measurements were used in the other studies [14, 19, 23]. Therefore, confirmation of the presence and location of the QTL is still needed, by performing a confirmation experiment.

Confirmation experiments have been described in several studies. In a backcrossing experiment in the pig, the presence of one or more QTL for fatness and growth was confirmed on pig chromosome 4 [18]. Furthermore, a grand-granddaughter design in dairy cattle has been used successfully to confirm QTL affecting milk yield [1, 5]. Another example to confirm QTL is the use of an advanced intercross line (AIL). In mice, for example, this resulted in the confirmation of QTL found in an earlier study [15, 29, 30]. Basically, an AIL is a population used for the fine-mapping of a QTL region and it is created by repeated intercrossing for a number of generations [7]. Because it is most suited for animals having a short generation interval, the AIL approach can be used in chickens.

The current report describes the use of an AIL to confirm the results of an earlier QTL mapping study. Therefore, from the previously used three-generation population [16] a generation 9 (G_9) population was produced by random intercrossing in each generation from generation 2 (G_2) onwards. The results of the analysis of the G_9 population are presented in the present study.

2. MATERIAL AND METHODS

2.1. Experimental population and observations

The three-generation full sib half sib design described by Van Kaam *et al.* [26] was used in a previous study to detect QTL affecting fatness [16]. The G_2 animals of this design were used to produce a G_9 population by random intercrossing in each generation. The population structure and number of animals are given in Table I. In total, 12 full sib G_8/G_9 families were produced with on average 84 offspring. The G_9 population consists of 546 male and 460 female animals.

The G_9 animals were raised in 15 hatches and housed in a litter system for broilers. Animal density was around 20 animals/m^2. The animals were in the same pen starting from day 0, where they received feed and water for *ad libitum* consumption and illumination was 23 h a day. A commercial broiler feed containing 12 970 $kJ \cdot kg^{-1}$ was used.

The birds were weighed at 5 weeks of age (BW5) and again at slaughter when they were 7 weeks of age (BW7). Around this age, commercial broilers reached the slaughter weight of 2 kg. After slaughter, the weight of the abdominal fat pad (AFW) was measured and the percentage of abdominal fat (AF%) was calculated. In addition, intramuscular fat content of part of the breast muscle, *pectoralis minor* was determined by means of extraction (Soxhlet method; NEN-ISO 1444 [9]) and the percentage of intramuscular fat (IF%) was calculated.

2.2. QTL regions

In the previous QTL analysis, QTL for AFW and AF% were found on chromosomes 1, 2, 3, 4, 7, 11, 13, 15, 18 and 27 [16]. From the results of this analysis six chromosomes were chosen for further analysis in the G_9 population. Chromosomes 1, 4, 15, and 18 were selected because they showed significant evidence for QTL for both AFW and AF%. All other chromosomes had a suggestive QTL for either AFW or AF%, therefore only the most promising chromosomes (chromosomes 3 and 27) were selected.

2.3. Genotyping

Genotyping of the microsatellite markers was done as described previously [6]. PCR amplifications were carried out in 12 μL reactions containing 10−60 ng genomic DNA, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl

Table I. Population structure and number of animals used for breeding, genotyping, and phenotyping.

Generation[1]	Number of animals		
	Breeding	QTL analysis	
G_0	28		
G_1	20	20	genotyped
G_2	71	456	genotyped
G_3	105	5363	phenotyped
G_4	122		
G_5	93		
G_6	108		
G_7	127		
G_8	100	24	genotyped
G_9	-	1006	genotyped & phenotyped

[1] G_0, etc. = Generation 0, etc.

(pH = 8.3), 1 mM tetra-methylammoniumchloride (TMAC), 0.1% Triton X-100, 0.01% gelatin, 0.2 mM each dNTP, 0.25 U Silverstar polymerase (Eurogentec, Liege, Belgium) and 2.3 pmol of each primer, one of which was labeled with a fluorescent dye (FAM, TET and HEX) at the 5′ end. The amplification reactions were as follows: 5 min 95 °C followed by 35 cycles of 30 s 94 °C, 45 s at 50 or 55 °C and 90 s at 72 °C, followed by a final elongation step of 10 min at 72 °C. Per animal PCR amplification products for 7 to 10 markers were combined and analyzed simultaneously on a 6% denaturing polyacrylamide gel, Sequagel-6 (National Diagnostics, Atlanta, GA, USA) on an ABI377 automatic sequencer (Applied Biosystems, Perkin Elmer, Foster City, CA, USA). Electrophoresis was performed for 2 h on 12 cm gels, and the results were analyzed using the Genescan and Genotyper software (Applied Biosystems, Perkin Elmer, Foster City, CA, USA).

A set of eight microsatellite markers was used to check the parentage from G_2 to G_9. For the QTL analysis, genotypes for 12 G_8/G_9 full sib families (1030 animals) were determined with 22 microsatellite markers. These 22 markers were located on chromosomes 1, 3, 4, 15, 18, and 27. The linkage map used in the present study was calculated with CRIMAP [11]. Further analyses were performed, using the recombination fractions obtained from CRIMAP [11] which were transformed to Haldane map distances [13] in the homemade QTL analysis program [26]. More information on the marker data is given in Table II.

Table II. Chromosomes and microsatellite markers that were used for the full sib QTL analysis in G_8/G_9. Map distances are given in cM on the Haldane scale.

Chromosome	Microsatellite markers	Map distance (cM)
1	*MCW0044*	0
	MCW0289	8.7
	MCW0297	16.3
	ADL0364	23.8
	ADL0359	44.6
	MCW0018	73.6
	MCW0058	114.6
	MCW0101	122.2
3	*MCW0116*	0
	MCW0148	2
	MCW0037	3.1
4	*LEI0122*	0
	MCW0276	63.6
15	*MCW0031*	0
	LEI0120	6.4
	MCW0052	28.7
18	*MCW0045*	0
	MCW0217	25.5
	ADL0290	36.7
27	*MCW0076*	0
	MCW0328	11.2
	ADL0376	23.6
		Total: 277.9

2.4. QTL analysis

Full sib QTL analysis was conducted using the regression interval mapping methodology as described by Van Kaam *et al.* [26] in which a single QTL was fitted. The analysis is an across family weighted full sib regression analysis. Because marker-QTL linkage phase can differ between families, QTL analysis was nested within families. Phenotypic values of G_9 animals were regressed on the probabilities of inheriting the first allele of each G_8 parent. Phenotypic

values were adjusted for fixed effects sex (2 classes) and week of hatching (15 classes). In the model, the family mean was included in order to account for polygenic differences between families. The model to fit a QTL at position k was:

$$y_{ij} = f_i + b_{s,ik}\, x_{s,ijk} + b_{d,ik}\, x_{d,ijk} + e_{ijk}$$

where: y_{ij} = the average adjusted progeny trait value for G_9 chicken j of family i; f_i = the polygenic effect of family i; $b_{s,ik}$ = the regression coefficient for the sire(s) of family i at position k; $x_{s,ijk}$ = the probability that the G_9 chicken j in family i at position k received the chromosomal segment from haplotype 1 from the sire; $b_{d,ik}$ = the regression coefficient for the dam (d) of family i at position k; $x_{d,ijk}$ = the probability that the G_9 chicken j in family i at position k received the chromosomal segment from haplotype 1 from the dam; e_{ijk} = the random residual.

Test statistics were calculated at each centimorgan, in order to test for the presence of QTL effects *vs.* the absence of QTL effects. The test statistic was the ratio of the explained mean square of the QTL effects in the numerator and the residual mean square of the full model in the denominator.

2.5. Significance thresholds

Chromosome-wise significance thresholds were calculated using the method of permutation testing [4]. This is an empirical method, which accounts for the distribution of the marker and phenotypic data. For each trait, 10 000 permutations at 1 cM intervals for each chromosome were performed. Considering that six independent chromosomes were analysed and assuming the number of significant chromosomes to follow a binomial distribution, the required threshold on the chromosome level Pc is such that $6P\text{c} = 1$, *i.e.* $P\text{c} \sim 0.16$. For claiming significant linkage, we applied the 5% experiment-wise significance level. Experiment-wise significance levels, taking into account testing of all the chromosomes, were calculated as: $P\text{g} = 1 - (1 - P\text{c})^{1/r}$, where ($r$) was obtained by dividing the length of a specific chromosome by the total length of all chromosomes. This is computationally more efficient than performing experiment-wise permutations, because in the current approach permutations only have to be performed for chromosomes that show evidence for QTL.

3. RESULTS AND DISCUSSION

3.1. QTL analysis G_8/G_9

From the results of the QTL analysis for AFW and AF% in the previous study [16], chromosomes 1, 3, 4, 15, 18, and 27 were chosen for further analysis in the G_9 population.

For the QTL analysis, three sets of a total 22 microsatellite markers were used on 12 G_8/G_9 full sib families (1030 animals) resulting in over 22 000 genotypes. These markers were only used to confirm the presence of the previously detected QTL in the AIL. Where possible, for each QTL region, three microsatellite markers were chosen, *i.e.* two at the border of the 95% confidence interval (CI) of the QTL and one in the middle. However, on chromosome 3 the three microsatellite markers were situated much closer together than the 95% CI and on chromosome 4 only two microsatellite markers were used. The QTL region on chromosome 1 was approximately four times as big as the other QTL regions, therefore more markers (eight) were chosen. For the selected chromosomal regions the marker order and map distance in cM on the Haldane scale [13] is shown in Table II. Marker order is the same as in the consensus linkage map reported by Groenen *et al.* [12] and map distances recalculated for cM on the Kosambi scale [17] are comparable to those in the consensus linkage map.

The results of the full sib QTL analysis are summarized in Table III. QTL were detected for at least one of the traits AFW, AF%, BW5, BW7, and/or IF% on chromosomes 1, 3, 15, and 27. For chromosomes 4 and 18, test statistics did not exceed the significance threshold for any of the traits measured in this experiment. On chromosome 1 two distinct QTL regions were identified (Fig. 1). In the region 0−30 cM, suggestive evidence was found for AFW and AF% and in the region 40−120 cM, significant evidence was found for BW5 and BW7 and suggestive evidence for AFW, AF%, and IF%. Suggestive QTL were found on chromosome 15 for BW5 and on chromosome 27 for AF%, BW5, and IF%. Furthermore, evidence on the chromosome-wise level was found on chromosome 3 for AFW, AF%, and BW7.

3.2. Power of the QTL analysis

Power was calculated using the methods described by Van der Beek *et al.* [25], assuming a QTL heterozygosity of 0.5 and an average distance between informative markers of 20 cM. In the three-generation experiment,

Table III. QTL for abdominal fat weight (AFW), percentage abdominal fat (AF%), body weight at 5 (BW5) and 7 (BW7) weeks of age, and percentage intramuscular fat (IF%) in the G_8/G_9 population of chickens derived from a broiler × broiler cross. Positions are given in cM on the Haldane scale.

Trait	Chromosome	Position (cM)	Marker bracket	F-ratio	*P*-value[1]
AFW	1	10	*MCW0289-MCW0297*	1.559	0.23†
		82	*MCW0018-MCW0058*	1.882	0.052^{c,†}
	3	0	*MCW0116-MCW0148*	1.430	0.062^{c}
AF%	1	11	*MCW0289-MCW0297*	1.474	0.29†
		84	*MCW0018-MCW0058*	1.732	0.11^{c,†}
	3	0	*MCW0116-MCW0148*	1.364	0.086^{c}
	27	11	*MCW0076-ADL0376*	1.848	0.016^{c,†}
BW5	1	68	*ADL0359-MCW0018*	2.135	0.018^{c,*}
	15	19	*LEI0120-MCW0052*	2.027	0.0093^{c,†}
	27	9	*MCW0076-MCW0376*	1.494	0.089^{c,†}
BW7	1	83	*MCW0018-MCW0058*	2.282	0.0061^{c,*}
	3	0	*MCW0116-MCW0148*	1.397	0.080^{c}
IF%	1	114	*MCW0018-MCW0101*	1.464	0.30†
	27	23	*MCW0328-ADL0376*	1.750	0.036^{c,†}

[1] Chromosome-wise *P*-value; ^{c} chromosome-wise linkage; * experiment-wise significant linkage; † experiment-wise suggestive linkage.

the power of the design to detect a QTL with an effect of $0.3\sigma_p$ is approximately 0.33 with α as 0.05. Using the same parameters, the power in the two generation (G_8/G_9) full sib design was 0.85, showing that the design of the present study was very powerful and that true and false QTL should be distinguishable. Nevertheless, one may fail to confirm the QTL in a subsequent experiment for several reasons as indicated by Marklund *et al.* [18]. For example the original observation may be a type I error or a large QTL effect may be caused by several linked QTL each with a small effect, and the linkage may break up in subsequent generations.

In the present study we consider a suggestive QTL from the previous study to be confirmed when in the present study the test statistics exceed the significance threshold for chromosome-wise linkage or suggestive linkage. A comparison between the results of both studies is shown in Table IV.

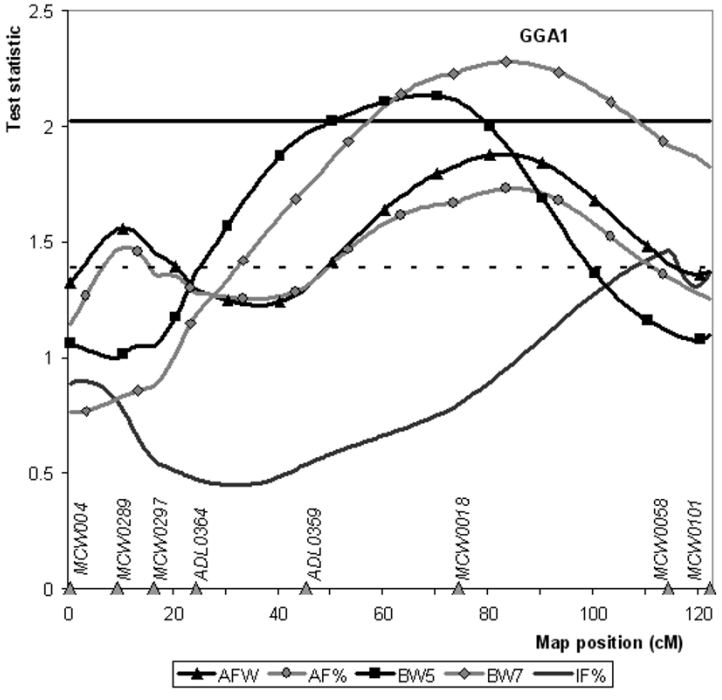

Figure 1. Test statistic values from the full sib QTL analysis for abdominal fat weight (AFW), percentage abdominal fat (AF%), body weight at the age of 5 and 7 weeks (BW5 and BW7), and percentage intramuscular fat (IF%) on chicken chromosome 1. Average thresholds for significance linkage at the 5% level (——) and for suggestive linkage (- - - -) are included. Map positions are given in cM on the Haldane scale.

3.3. QTL for abdominal fatness

On chromosome 1 the QTL for AFW as well as AF% were confirmed (Tab. IV). Moreover, for both traits the analysis revealed two distinct peaks on this chromosome at a distance of around 75 cM (Fig. 1). An additional two QTL regression analyses were undertaken by fitting two QTL for both AFW and AF%. Significance levels were obtained by two dimensional permutation to test for the presence of two QTL *versus* one QTL. The significance level was adjusted to the experiment-wise level using the same adjustment as applied in the single QTL analysis. The results of this analysis suggest that two distinct QTL for fat deposition are present on this chromosome ($Pg < 0.08$ for AFW and $Pg < 0.16$ for AF%). The two QTL (the first between *MCW0289-MCW0297* and the second between *MCW0018-MCW0058*) are suggestive for both AFW and AF%. Support for the QTL between *MCW0289-MCW0297* is

Table IV. Significance levels for QTL regions included in the G_8/G_9 population. Traits are abdominal fat weight (AFW) and percentage abdominal fat (AF%) at 7 weeks of age, body weight at 5 (BW5) and 7 (BW7) weeks of age, and percentage intramuscular fat (IF%) at 7 weeks of age.

Chr	AFW		AF%		BW5		BW7		IF%	
	G_2/G_3	G_8/G_9	G_2/G_3	G_8/G_9	G_2/G_3	G_8/G_9	G_2/G_3	G_8/G_9	G_2/G_3	G_8/G_9
1	†[1]	c,†	*[1]	c,†	nd	c,*	-	c,*	nd	†
3	-	c	†[3]	c	nd	-	-	c	nd	-
4	†[2,3]	-	†[2]	-	nd	-	-	-	nd	-
15	†[1]	-	†[1,2,3]	-	nd	c,†	-	-	nd	-
18	†[1]	-	†[1]	-	nd	-	-	-	nd	-
27	-	-	†[2]	c,†	nd	c,†	-	-	nd	c,†

c Chromosome-wise linkage; * significant linkage; † suggestive linkage; nd not determined. [1] Effect detected at 10 weeks of age. [2] Effect detected at 9 weeks of age. [3] Effect detected at 7 weeks of age.

also given by Ikeobi *et al.* [14], who reported a QTL for abdominal fatness, which co-locates with ours. The 95% CI (determined by bootstrapping) of the first and second QTL are 25 and 60 cM, respectively. This is considerably smaller than the CI of the G_2/G_3 QTL (~145 cM). The reduction of the CI is due to the increased number of informative meioses as a result of the larger number of animals used in the G_9.

The suggestive QTL for AF% on chromosomes 3 and 27 were also confirmed in this study and evidence at the chromosome-wise level for AFW was detected on chromosome 3. The QTL on chromosome 27 is supported by McElroy *et al.* [19] who found a suggestive linkage for fat weight ($P = 0.06$) with a single marker (*MCW0233*), close to our QTL. For the QTL region on chromosome 3, no other fatness QTL have been reported in the literature.

The suggestive QTL for AFW and AF% previously found on chromosomes 4, 15, and 18 could not be confirmed, suggesting that the previously found QTL were falsely identified. However, power was calculated using a marker distance of 20 cM, while on chromosome 4 marker distance is 63.6 cM (Tab. II). Therefore, power to detect a QTL on this chromosome with an effect of $0.3\sigma_p$ is approximately 0.52 with α is 0.05. This is considerably lower than the previous calculated power of 0.85, as is the chance to detect a QTL. Therefore, we cannot completely exclude the possibility of the presence of a QTL for abdominal fatness on chromosome 4. Furthermore, Ikeobi *et al.* [14] found significant QTL for fatness traits on chromosome 15 in the same region as the

previous identified QTL in the G_2/G_3 population [16]. These results suggest that there might be genes located on this chromosome, which are involved in the regulation of fat deposition.

3.4. QTL for body weight

Evidence for QTL for BW5 and/or BW7 was found on chromosomes 1, 3, 15, and 27 (Tab. III). In the three-generation design of our previous study [16] we did not find any evidence for the presence of QTL with an effect on BW on these chromosomes. However, in our group, several QTL studies were performed using the same three generation design, with the same genetic background, but different G_3 offspring [26–28]. In two of these studies, a suggestive QTL for BW7 was identified on chromosome 1 near microsatellite markers *MCW0058* and *LEI0071* [26, 27], which is confirmed by the results of the present study.

The presence of QTL for BW on chromosomes 1, 15 and 27 is supported by the results of several other QTL studies. In the same region on chromosome 1 Tatsuda and Fujinaka [24] detected QTL for BW at 13 and 16 weeks of age. Furthermore, Carlborg *et al.* [3] found QTL on chromosomes 15 and 27, whereas, Sewalem *et al.* [21] did on chromosomes 1 and 27.

3.5. QTL for intramuscular fat

In addition to the traits AFW, AF% and BW, which have been analyzed in several QTL studies, IF% was also analyzed in this study. So far no QTL mapping studies have been conducted for this trait in poultry. We found suggestive evidence for IF% on chromosomes 1 and 27. Considering the fact that on chromosomes 1 and 27 evidence was also found for BW, AFW and/or AF% (Tab. III; Fig. 1), it is likely that the underlying gene has pleiotropic effects. The estimation of genetic parameters on the present data [31] showed that IF% and BW were genetically highly correlated (0.87−0.91), whereas the genetic correlation between AF% and IF% was negative (−0.31) and between AFW and IF% it was almost zero (0.02). These correlations suggest [10] that the metabolic pathways for growth and fat deposition in the muscles are influenced by the same genes in the same direction, whereas the metabolic pathway for fat deposition in the abdomen might be influenced by these genes in the opposite directions or even by other genes.

4. CONCLUSION

The results of this study show the confirmation of QTL found in an earlier generation in an AIL. Moreover, on chromosome 1 we were able to identify two distinct regions for fat deposition. This is the first step towards the fine mapping of the QTL for fat deposition. Nevertheless, the identification of conserved chromosomal segments (*i.e.* haplotype blocks), which are associated with the observed QTL will be needed to further reduce the size of the QTL regions. To identify these haplotype blocks more densely spaced markers are needed. Therefore, new markers (*i.e.* SNPs) need to be developed. This is an essential step before moving towards the next phase of identifying the underlying genes responsible for the observed QTL.

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