



## QTL FOR EGG QUALITY

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### Abstract

Poor egg quality causes economic losses at all production stages and leads to increased risk of pathogen contamination. Improving the quality traits by traditional selection has been difficult and direct selection based on genetic markers might greatly enhance the breeding of egg quality traits. This paper describes recent advances in mapping and characterization of quantitative trait loci (QTL) affecting egg quality in egg layers. In addition to reviewing the results from literature, recent results on mapping QTL for egg shell strength within the SABRE project (FOOD-CT-2006-016250; Cutting edge genomics for sustainable animal breeding) are presented.

**Keywords:** layer, egg quality, QTL mapping, SNP, marker

### Introduction

The two economically most important hereditary egg quality characteristics are the strength and integrity of the eggshell and the quality of the egg white. Broken eggshells may cause production losses up to 10 % and also provide a route for pathogen contamination. Yolk and albumen quality are important for processed egg products. Thinning of the egg white exposes the egg to microbial infections due to altered structure and composition. Other important quality factors of interest for the consumers, such as olfactory characteristics or shell colour, also have genetic components. Improving the quality traits by traditional selection has been difficult, as the phenotypic measurements are difficult or time-consuming and in some cases unfavourable genetic correlations exist with other important production traits.

Recent advances in the availability of genomic information have made the dissection of the hereditary variation behind these traits possible. The first genome scans to identify loci affecting egg quality traits have been based on medium-density microsatellite maps. Quantitative trait loci (QTL) have been reported for various eggshell characteristics and correlated bone traits, such as bone density and mineral content. Less emphasis has been put on egg white quality, olfactory characteristics, or eggshell colour. Similar QTL on specific chromosomal areas have been supported by several studies. However, their locations have not yet been refined with useful accuracy for population level marker-assisted selection (MAS).

The quantitative trait loci identified and the experimental designs used in the chicken have been reviewed by Abasht et al (2006). All significant QTL have been collected in the Chicken Quantitative Trait Loci database (<http://www.animalgenome.org/QTLdb/chicken.html>). The majority of the 791 chicken QTL in the database are affecting growth or carcass traits, and only 113 are reported for egg quality traits. The database also includes tools to link the QTL data to other types of genomic information, such as radiation hybrid maps, physical maps, linkage maps, comparative maps to the human genome and SNP information aligned against the chicken



genome (Hu et al. 2007). These tools greatly facilitate further characterization of the QTL regions.

When a QTL region with potentially interesting genetic variation has been identified, the next step is fine-mapping the region to identify tightly associated marker loci for MAS or positional candidate genes that could be searched for relevant sequence variation. Until now, only few fine-mapping studies have provided suitable markers for selection or information of genes behind the variation. Not more than one gene mutation causing a quality defect is presently known (fishy taint in brown egg layers, Honkatukia et al. 2005). Before the results of the fine-mapping can be used for selection, it is necessary to estimate the pleiotropic effects of the QTL on other economically important traits. In addition, one should be aware of the possibility of epistatic effects between loci, i.e. the different outcome of allelic effects with different genetic backgrounds. Including epistatic effects in QTL analyses may also help in uncovering novel QTL and their interactions.

MAS using linked markers may not be directly applicable across lines as the linkage phase between QTL alleles and marker alleles may vary. Gene assisted selection (GAS), based on the identified causative mutations can be used directly in breeding programmes to increase the frequency of favourable alleles or to eliminate unfavourable ones. The first example of GAS used for improving egg quality is the use of FMO3 markers by Lohmann Tierzucht GmbH for eliminating fishy egg taint from brown layer lines.

### **QTL for eggshell quality**

Regions affecting eggshell quality have been found on chromosomes 1, 2, 4, 5, 7, 12 and Z (Abasht et al. 2009, Sasaki et al. 2004, Schreiweis et al. 2006, Tuiskula-Haavisto et al 2002, Wardecka et al. 2002). In addition, several QTL affecting bone strength, mineral density and mineral content are possibly related with the process of recruitment of calcium reserves for eggshell formation (see e.g. Dunn et al. 2007). The mapping populations as well as phenotypic measurements (eggshell thickness, eggshell strength, eggshell weight, eggshell percentage, egg shape, specific gravity and different bone measures) have varied among studies and it is not therefore clear which of the findings may reflect the action of same loci. No fine-mapping studies have been reported for these traits yet.

Within the SABRE project (FOOD-CT-2006-016250; Cutting edge genomics for sustainable animal breeding), the Work Package 7 (Product Safety) aims at fine-mapping of QTL affecting egg shell quality, testing associations of candidate gene SNPs in commercial populations and doing transcriptomic profiling of genes and gene pathways involved in egg shell formation.

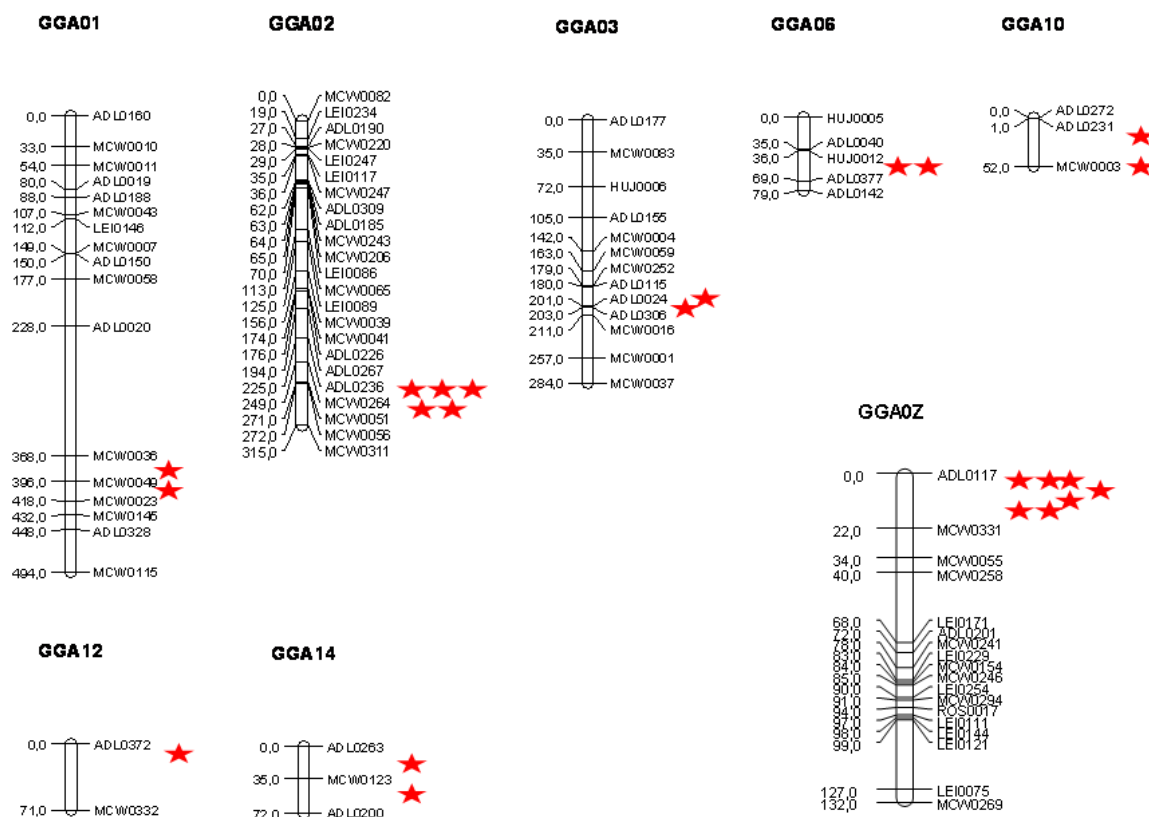
A reciprocal F<sub>2</sub> cross between two commercial pedigree lines (Lohmann Tierzucht GmbH breeding program) with 1600 F<sub>2</sub> hens is used to characterize quantitative trait loci affecting eggshell quality traits. The genome regions to be fine-mapped with a denser map were decided based on the results of a genome scan using 162 microsatellite markers located on 27 chromosomes (no informative markers were found on 11 microchromosomes). A subset of the F<sub>2</sub> population (668 F<sub>2</sub> hens and their 48 parents and 44 grandparents) was genotyped in the first phase. The calculated linkage maps cover 2585 cM.

The phenotypes were measured as averages of measurements from tree consecutive eggs at each covered age. The data was corrected for hatch and cage

effects. Egg weight (g) was measured at the age of 30 weeks and shell weight (g) at the age of 42 weeks. Breaking strength (kp) and deformation (mm) were measured with a Canadian Egg Shell tester at 35, 40, 50 and 70 weeks of age. Traits “deformation average” and “breaking strength average” were formed as the weighted average from 35, 40 and 50 week measurements.

Autosomal linkage groups were first analysed using an additive and dominance model with QTLEXPRESS to map QTL affecting these traits. The analyses were extended by using the epistasis module in GridQTL (<http://qtl.cap.ed.ac.uk/>). Sire effects were fixed in the search models for all traits. In addition, biologically correlated traits were fitted as covariates for certain traits (e.g. egg weight was a covariate for shell weight). A nested search algorithm was used to detect epistatic QTL: 1) using the model of (Q1+Q2+Q1Q2) to search for a best pair (with the minimum residual sum squares); 2) calculate the residual sum squares of the model of (Q1+Q2) with the QTL positions identified in 1); 3) derived F statistics and test significance. The Z chromosome was analysed via a linear regression program where only additive effects could be analysed.

In all, 23 QTL affecting eggshell strength were found in the genome scan (Figure 1). The results confirm findings on chromosomes 1, 2, and Z from other studies. The mainly additive effects of each of the QTL explain 2 –5 % of the phenotypic variance. Genome-wide significant QTL were found on chromosomes 2, 6 and 14, and additional chromosome-wise significant QTL seem to cluster on these chromosomes and on chromosome 3. At these regions with QTL for the same trait at several production ages the results seem very convincing. For example, on chromosome 2, QTL affecting shell breaking force (at 35 and 40 weeks of age) and QTL affecting shell deformation (average and at 35 and 40 weeks of age) were identified within the marker bracket ADL0236 - MCW0264. On the Z-chromosome, a cluster of QTL affecting both eggshell breaking strength and deformation was found within the marker interval ADL177-MCW0331.



**Figure 1.** QTL affecting egg shell strength (breaking force and deformation) identified in a genome scan of commercial layer lines within the SABRE project. Only linkage groups with significant findings are shown. The marker bracket locations of the QTL are indicated with stars.

All traits, including production traits, were analyzed for epistatic QTL effects. No epistatic QTL effects were found for egg quality traits. One genome-wide epistatic pair was detected for body weight at 30 weeks of age. The loci were located on chromosome 1 at 155 cM and chromosome at 8 cM. The body weight epistatic pair is similar to one of those reported by Carlborg et al. (2004) for early growth. Thus it seems that epistatic effects do not play a significant role in the genetics of eggshell quality in this mapping population, but that we can confirm epistatic effects on growth.

The five most interesting regions on chromosomes 2, 3, 6, 14, and Z are currently being fine mapped by densely spaced SNP markers (approximately 3 SNPs per cM). Information on gene expression differences in the shell gland vs. other parts of the oviduct and in the shell gland before and during egg formation (Joel Gautron, INRA) will be combined with the fine mapping results to identify potential candidate genes in the QTL regions. At the final stage, potentially important SNP effects will be tested in commercial populations.

### **Egg white quality**

Egg white quality QTL (Haugh units) have been mapped to chromosomes 1 and 2 (Hansen et al. 2005, Tuiskula-Haavisto et al. 2002). The attempt to fine-map the major QTL affecting HU in the White Leghorn X Rhode Island Red mapping population (explaining 7% of the phenotypic variation) (Tuiskula-Haavisto et al. 2002) resulted in the splitting of the QTL region to two distinct QTL regions and excluding one potential candidate gene, vimentin, within the original QTL region (Honkatukia et al. 2005). Recently effects on egg white quality were assigned by SNP association analysis to chromosomes 5, 18 and Z (early albumen height) and 3, 5, 19 and 23 (late albumen height) (Abasht et al. 2009).

### **Olfactory characteristics**

Honkatukia et al. (2005) have shown that fishy odour in brown eggs is caused by a missense mutation in the chicken FMO3 gene, located on chromosome 8. The mutation is associated with elevated trimethylamine (TMA) levels (after feed containing TMA precursors) in several brown egg layer lines. The mutation causes an amino acid substitution in an evolutionary highly conserved motif of the monooxidase enzyme, leading to improper oxidation of the odorous TMA. A QTL for egg “after taste” has also been mapped to a distinct location in the middle of chromosome 8 and a QTL for egg odour to the linkage group E22C19W28 (Wright et al. 2006).

### **Conclusions**

Several QTL with effects on egg shell quality have been identified and are currently being fine-mapped within the SABRE project. Although their individual effects are small, their combined effects may add up to 20% of total genetic variation in egg shell strength in this population. However it is clear that other variation exists in other populations, so globally the proportion explained is small. Furthermore, not all chromosomes have so far been covered by microsatellite markers. In the very near future the use of dense chicken SNP panels in genome wide association analyses will give a more comprehensive understanding of the genetic architecture of egg quality.

Epistatic interactions between genes seem not to play a significant role in egg shell quality, in contrast to findings related to early growth. This facilitates the further characterization of these QTL as well as the use of associated markers in selection.



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