

Effect of OCX-32 and SIBLING Gene Complex on Shell and other Egg Quality Traits in White Leghorn lines

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Summary

The improvement of both quality and safety of eggs has been an important breeding objective for primary egg breeding companies. Technological advances allow precise measurement of internal and external traits that can be incorporated in breeding programs for improvement through genetic selection. Knowledge of genes with known effect can be incorporated to accelerate genetic progress. Records from two White Leghorn (WL) lines (1, n=978 and 2, n=823) were used. Individual egg quality traits were measured at two ages (1=59-64 and 2=81-86 wk). Data collected included: egg weight (EW, g), shell colour (CO), shell quality (breaking strength (BS, gF), dynamic stiffness (KDyn) and incidence of micro-cracks (MCr)), and internal egg quality (albumen height (AH, mm), and yolk weight (YW, g). Samples of eggs were used for cuticle determination using attenuated total reflection-Fourier transformed infrared spectroscopy (ATR-FTIR) and shell ultra-structure using SEM. In addition, SNP variants resulting in protein changes in OCX-32 and SIBLING complex genes were identified within the two lines. Single SNP genotypes were generated and subsequent haplotypes within each region were defined. Association analyses were carried out with a linear model including hatch effect, and both the genotypic and the allele substitution effect models for variants within OCX-32, and SIBLING complex. Egg quality deteriorated with age in both lines, as expected. Older hens showed higher incidence of MCr, lower BS, and a marked reduction in the amount of cuticle. Shells from Line 1 had less cuticle coverage than shells for Line 2 at both ages. Significant effect of OCX-32 haplotypes on various egg quality traits were seen in both lines at both ages. The most important haplotype accounted for up to +159 gF of BS at 62 wk; and smaller eggs, with lower incidence of MCr, in older hens in Line 1. Similar effects but of less magnitude were found in Line 2. The SIBLING complex segregated only in Line 2, and showed significant association with KDyn, YW and CO (Age 1), and BS and CO (Age 2). Variation at SIBLING complex and OCX-32 was shown to affect shell quality traits in two unrelated WL lines at two ages. The amount of cuticle deposited differed among different variants of OCX-32 and SIBLING. Variants in both regions also affected other external and internal egg quality traits, implying complex roles of these genes in the overall process of egg formation.

Keywords: SNP, haplotypes, egg quality, layer chicken, association study

Introduction

Improving the quality and safety of eggs are important goals in layer genetics. This requires precise measurements and adequate selection tools, for eggshell quality traits. In addition to traditional quantitative methods, molecular techniques allow the identification of specific genes and their proteins involved in shell structure, composition and mineralization. For

example the role of the ovocalyxin-32 (OCX-32) gene and of the small integrin-binding ligand N-linked glyco-proteins (SIBLING) gene complex, which plays key roles in bone mineralization and bone remodelling (Staines et al., 2012) can be explored. The SIBLING's equivalent region of the chicken genome identifies a cluster containing four of these genes, whose expression has been found within the oviduct. This suggests significant involvement of this gene complex in eggshell formation. Variants of OCX-32 and SIBLING have been shown to be associated with shell quality traits. The SIBLING gene cluster found on chromosome 4, contains a high proportion of QTL influencing egg weight and relative size of various egg components.

The objective of this study was to establish associations between gene variants at the OCX-32 and SIBLING gene cluster with egg quality traits, particularly shell quality, using data from two unrelated White Leghorn lines.

Material and methods

Records from two White Leghorn lines from a single generation were used. Traits were measured at two ages: 62 (59-64) and 84 (81-86) weeks, and included: egg weight (EW, g), eggshell color (CO, index combining L*a*b parameters of the Konica Minolta Chromameter® system), and shell quality: breaking strength (gF, taken at the egg's equator (62 wk) and poles (84 wk), using the Futura FEST device; and Acoustic Egg Test (AET, both ages) to measure dynamic stiffness (KDyn) and incidence of micro-cracks using the Columbus apparatus (Octinion, Belgium). Internal egg quality traits included albumen height (AH, mm), and yolk weight (YW, g). Sub-samples of eggs (n= 175 and 304 for Lines 1 and 2, respectively) at both ages were used for cuticle determination using attenuated total reflection-Fourier transformed infrared spectroscopy (ATR-FTIR) and shell ultra-structure using scanning electronic microscopy (SEM) (Rodriguez-Navarro et al., 2013). SNP variants resulting in protein changes in OCX-32 and SIBLING complex genes were identified within the two lines. Single SNP genotypes were generated and subsequent haplotypes within each region were defined. For the SIBLING complex, haplotypes were determined within 3 genes (OPN, OC116 and IBSP); one SNP per gene, then grouped for the three genes to define SIBLING haplotypes.

Association analyses were carried out with a linear model including hatch effect, and both the genotypic and the allele substitution effect models for variants within OCX-32, and SIBLING complex.

Results and discussion

For Line 1, two haplotypes were found at OCX-32: H02 (freq = 0.76) and H07 (freq = 0.24). There was little segregation for the SIBLING gene cluster, so no haplotypes were defined. For Line 2, two haplotypes for OCX-32 were found, H05a (freq= 0.32) and H07 (freq = 0.68); while, there were multiple SIBLING haplotypes; but, two were at high enough frequency for analysis (Hap_111= 0.59) and Hap_HHH= 0.39). Hap_HHH is heterozygous for different proteins for all 3 genes within the SIBLING complex.

Egg quality deteriorated with age in both lines, as expected. Older hens (81 to 86 weeks) showed a higher incidence of micro-cracks, and lower breaking strength values (although the direction of the BS measurement differed with age). The dynamic stiffness values didn't differ between ages, in part due to the effect of egg size. There were also higher colour index values, higher grey spot scores and lower albumen height values in eggs from

older hens. A marked reduction in the amount of cuticle was observed in older hens. This was confirmed by a decreased of the ATR-FTRI signals associated with the cuticle components (amides, polysaccharides and lipids), and increased signals for carbonates. In general, eggs from Line 2 eggs had better shell ultra-structure, thicker shells, and greater cuticle coverage than those from Line 1 at both ages.

Significant effect of OCX-32 haplotypes on various egg quality traits were seen in Line 1 at both ages as summarized in Tables 1 and 2. The most important effect was for breaking strength, in favour of haplotype H07, which accounted for +159 gF in the equator (Age 1) and +146 gF at the poles (Age 2), per copy. This haplotype also produced smaller eggs, which had lower incidence of micro-cracks, especially in older hens. For Line 2, a significant effect of OCX-32 haplotypes and various egg quality traits were seen at both ages as summarized in Tables 1 and 2. The most important effect was for breaking strength. In this line, haplotype H05a showed better BS than H07. Homozygotes for the H05a haplotype had shells 98 gF stronger at the equator (Age 1) and 21 gF at the poles (Age 2) than heterozygotes with the H05a haplotype. This haplotype also was associated with smaller eggs and better albumen height at both ages. Associations at the SIBLING complex were significant for KDyn, shell colour and yolk weight at Age 1, and for breaking strength and micro-crack at Age 2. Hap_HHH had shells 61 and 203 gF stronger than Hap_111 at Ages 1 and 2.

Table 1. OCX-32 associations¹ with egg quality traits by line at age 1 (62 wk).

Line	Egg weight, g	Micro-crack, %	Kdyn, N/m	Breaking strength (equator), gF	Albumen height, mm	Shell color index	Yolk weight, g
1	**	*	ns	***	**	***	**
2	***	na	*	**	***	**	***

¹ ***: P<0.0001; **: P<0.01; *: P<0.05; §: P<0.10; ns, P>0.10

Table 2. OCX-32 associations with egg quality traits by line at age 2 (84 wk).

Line	Egg weight, g	Micro-crack, %	Kdyn, N/m	Breaking strength (poles), gF	Albumen height, mm	Shell color index
1	***	***	***	***	***	***
2	**	ns	ns	**	***	***

In avian species, expression of the SIBLING complex genes has been found within the oviduct suggesting roles of these proteins in eggshell formation (Hincke et al., 1999; Rose and Hincke, 2009; Miksik et al., 2007). Variation within these genes has been associated with numerous shell quality traits including shell thickness, shell elasticity and stiffness (Dunn et al., 2008). In the chicken, this gene cluster is found on chromosome 4, which contains a high proportion of QTL influencing egg weight and relative size of various egg components (Wolc and Fulton, 2017). These multiple sources of information suggest that variation within the cluster of SIBLING genes is involved in variation in various shell quality traits. This was confirmed herein in Line 2, for dynamic stiffness at both ages; and in older hens for micro-

crack detection and breaking strength at the poles. Variants within OCX-32 were previously associated with egg quality traits in a study with multiple lines of layers (Fulton et al., 2012). Results herein confirm the influence of OCX-32 variants on egg quality utilizing different eggshell quality measurements. A consistent effect of OCX-32 variants was observed on breaking strength in the two lines, ages and the two directions of measurement. In addition, there was an effect on micro-crack detection (Line 1), with an increase in magnitude as hens aged, as expected. Dynamic stiffness was also influenced by OCX-32 in Line 1 (older age) and Line 2 (younger age). In addition to these effects on shell quality, variants at both genes also affected other external and internal egg quality traits, implying complex roles of these genes in the overall process of egg formation. The amount of cuticle deposited differed among different variants of OCX-32 and SIBLING at both ages. Association analyses for specific chemical components are summarized in Tables 5 and 6 for younger hens. OCX-32 significantly influenced signals for OH, lipids and sugar in Line 1, and sulphates and sugar in Line 2. Variation within the SIBLING complex significantly influenced signals for sulphates and sugar in Line 1.

Table 3. SIBLING complex associations with egg quality traits at age 1 (62 wk).

Line	Egg weight, g	Micro-crack, %	Kdyn, N/m	Breaking strength (equator), gF	Albumen height, mm	Shell color index	Yolk weight, g
2	*	na	* **	ns	ns	***	***

Table 4. SIBLING complex associations with egg quality traits at age 2 (84 wk).

Line	Egg weight, g	Micro-crack, %	Kdyn, N/m	Breaking strength (poles), gF	Albumen height, mm	Shell color index
2	ns	*	*	***	ns	ns

Table 5. OCX-32 associations with cuticle chemical composition by line at age 1 (62 wk).

Line	OH, %	AmidesI-II, %	CO ₃ , %	Lipids, %	Sulphates, %	Sugar, %
1	*	ns	ns	*	ns	**
2	ns	ns	§	ns	*	*

Table 6. SIBLING associations with cuticle chemical composition in line 2 at age 1 (62 wk).

Line	OH, %	AmidesI-II, %	CO ₃ , %	Lipids, %	Sulphates, %	Sugar, %
1	§	ns	§	ns	*	**

Some of the described haplotypes were found at relatively high frequencies. If selection were applied on a single trait (i.e., shell quality) then the favourable haplotype for shell quality would be fixed. Or, alternatively if there were two favourable haplotypes then both would be present and one may be at a higher frequency than the other just due to chance.

However, in the actual breeding program, selection is applied on multiple traits, related to production, quality and efficiency; therefore, frequency changes are influenced by correlations among traits. The haplotype association of better shell quality with smaller eggs is a good example. Selection is based not on the actual haplotypes, but on the overall phenotype, which is composed of multiple inter-related traits which can prevent rapid haplotype fixation.

The SIBLING complex was close to fixation in one of the lines, so while we could not specifically test it, it is likely a SIBLING haplotype is favourable for multiple of traits included in the breeding program.

Conclusion

Variation at both the SIBLING complex and OCX-32 was shown to affect shell quality traits in two unrelated WL lines at two ages. Variants in both regions also affected other external and internal egg quality traits, implying complex roles of these genes in the overall process of egg formation.

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