Quantitative Trait Loci Mapping for External Egg Traits in F₂ Chickens

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Quantitative trait locus (QTL) mapping of external egg traits in chickens was carried out with 143 microsatellite markers using 388 F₂ hens obtained from an intercross of a Japanese Large Game (Oh-Shamo) male and three White Leghorn females. External egg traits, such as the weight and length of eggs and the weight, thickness, strength, and color of eggshells were measured at three different stages of egg production, *i.e.* early, middle, and late stages. QTL analyses revealed 10 significant and three suggestive QTLs with main effects on external egg traits on chromosomes 1, 2, 4, 5, 8, 10, 11, 12, 17, and Z. The QTL alleles derived from Oh-Shamo, one of the indigenous Japanese breeds, uniquely increased most external egg traits, whereas some QTL alleles had opposite effects on phenotypic differences between Oh-Shamo and White Leghorn. The QTLs detected accounted for 3.09–15.39% of the phenotypic variances. Although the QTLs on chromosomes 5, 8, and Z were found throughout all three stages, the others were identified at only one or two stages. Epistatic QTLs for external egg traits were not detected in the present study. The age-related changes of our main-effect QTLs illustrate one aspect of the complex genetic basis of external egg traits.

Key words: age-related change, chicken, external egg quality, mapping, quantitative trait loci

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Introduction

A great number of researches have been conducted to understand the genetic basis of quantitative traits which are controlled by multiple quantitative trait loci (QTLs), environment, and their interactions (Mackay *et al.*, 2009). It is important to identify the chromosomal regions of QTLs affecting such economically important traits, because such positional information will be useful to improve the livestock breeding performances in the future. In chickens, many QTL mapping studies have detected loci associated with growth, egg, meat, behavior, and disease traits (Abasht *et al.*, 2006; Hu *et al.*, 2007, 2012).

External egg traits, which are directly related to the quality of egg products, can be defined as egg weight, egg size, eggshell color, eggshell strength, eggshell weight, and eggshell thickness (Fulton, 2004; Roberts, 2004). In addition, the structure and formation of the eggshell and the properties of

the eggshell matrix proteins have been investigated (Nys *et al.*, 2004; Hincke *et al.*, 2010). Dunn *et al.* (2009) reported that DNA polymorphisms in six eggshell-related genes, *ovocleidin-116*, *osteopontin*, *ovocalyxin-32*, *ovalbumin*, *estrogen receptor*, and *carbonic anhydrase II*, were associated with eggshell quality. However, these genes were accounted for only a part of the entire genetic variation of eggshell quality. Accordingly, it is very likely that many QTLs with main and epistatic effects on these traits remain undiscovered.

There have been some reports in which eggshell quality (Roland, 1979) and brown eggshell color (Odabasi *et al.*, 2007) are said to be influenced by age of hens. Hence, age-related changes of QTLs may have contributed to these egg traits. However, the previous QTL studies on egg traits focused on only one or two stages of egg production. In the present study, we measured external egg traits at three different stages of egg production, *i.e.* early, middle, and late stages, in an attempt to understand age-related changes of QTL expression.

In the present study, we used the Japanese Large Game (Oh-Shamo) and White Leghorn breeds as parental breeds for development of a QTL mapping population. The Oh-

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Table 1. Microsatellite DNA markers genotyped in the present study

Marker	Chromosome or linkage group	Map position (cM) ¹	Reference map position (cM) ²	Marker	Chromosome or linkage group	Map position (cM) ¹	Reference map position (cM) ²
MCW0248	1	0	19	ABR0017	8	0	0
LEI0209	1	24.5	56	MCW0305	8	27.3	15
MCW0010	1	35.1	72	MCW0095	8	37.2	26
MCW0111	1	67.6	118	ADL0345	8	56.2	56
ROS0327	1	70	118	ROS0075	8	95.9	94
ADL0188	1	79.1	133	ROS0078	9	0	0
ADL0124	1	84.8	143	MCW0329	9	42.6	54
LEI0068	1	91.1	151	MCW0135	9	52.2	61
LEI0146	1	102.5	169	ABR0526	9	79.2	*
MCW0112	1	132.4	205	ADL0259	9	93.5	122
MCW0058	1	157.8	241	MCW0134	9	104.4	132
LEI0101	1	172.4	259	ADL0272	10	0	44
LEI0198	1	256.7	364	ADL0106	10	19.3	88
MCW0036	1	282.3	386	LEI0112	10	37.5	107
LEI0106	1	319.2	426	ADL0123	11	0	22
MCW0102	1	336.8	441	ADL0210	11	22.5	54
ADL0245	1	362.7	459	MCW0066	11	35.5	69
ABR0328	1	367.5	475	ABR0037	11	57.5	90
GCT0032	1	407.3	518	ADL0372	12	0	0
MCW0107	1	460.3	565	ADL0044	12	61.9	44
LEI0124	2	0	*	MCW0340	13	0	2
MCW0205	2	9.1	2	LEI0251	13	46.9	47
MCW0184	2	73.8	60	ADL0214	13	79.7	70
ADL0190	2	75.6	62	MCW0104	13	81.1	74
ADL0176	2	133.6	116	MCW0296	14	0	0
MCW0062	2	182.5	172	MCW0123	14	45.2	45
LEI0096	2	230.8	233	ADL0263	14	68.2	72
MCW0173	2	240.6	243	ABR0517	14	81.5	*
MCW0027	2	251.2	255	ADL0206	15	0	0
LEI0147	2	270.5	282	MCW0031	15	1.5	7
MCW0185	2	286.8	302	MCW0211	15	54.8	49
LEI0237	2	302.5	320	ABR0530	17	0	*
LEI0070	2	363.8	379	ADL0293	17	14	26
ADL0146	2	387.6	403	MCW0330	17	27.7	41
MCW0261	3	0	0	ABR0387	17	32.2	*
MCW083	3	97.1	51	ADL0202	17	46.6	70
ADL0229	3	149.6	111	ABR0374	18	0	*
LEI0115	3	179.8	143	MCW0217	18	16.1	24
MCW0252	3	223.8	201	ABR0133	19	0	*
LEI0223	3	246.3	226	ABR0180	19	24	*
MCW0016	3	252.3	247	MCW0304	19	32.7	*
MCW0156	3	271.3	276	MCW0287	19	35.7	40
ADL0143	4	0	0	MCW0119	20	0	0
ADL0203	4	40.7	35	ADL0125	20	12.5	26
LEI0095	4	87.8	78	ADL0262	23	0	0
MCW0005	4	105.1	101	MCW0165	23	1.9	1
ADL0266	4	133.1	138	ROS0302	24	0	20
LEI0076	4	169.9	182	MCW0301	24	19.9	48
ABR0331	4	176.5	183	APOA1sts1	24	26.8	53
MCW0240	4	201.1	201	APOA1sts2	24	27	53
ABR0622	4	233.8	*	MCW0262	26	0	26
ABR0045	4	287.2	*	MCW0069	26	13.4	47
MCW0038	5	0	71	ABR0006	26	38.2	56
MCW0214	5	21.8	88	MCW0233	27	0	19
MCW0078	5	34.5	93	MCW0328	27	33	47
MCW0223	5	57.9	123	GCT0022	27	38.9	50

Table 1. (Continued)

Marker	Chromosome or linkage group	Map position (cM) ¹	Reference map position (cM) ²	Marker	Chromosome or linkage group	Map position (cM) ¹	Reference map position (cM) ²
<i>ADL0233</i>	5	77.6	151	<i>LEI0135</i>	28	0	0
<i>ADL0166</i>	5	91.9	162	<i>ADL0254</i>	28	20.9	25
<i>ROS0330</i>	5	121.9	198	<i>ADL0284</i>	28	23.5	25
<i>MCW0118</i>	6	0	*	<i>ADL0299</i>	28	55.4	60
<i>ABR0028</i>	6	18.9	*	<i>ABR0597</i>	32	0	*
<i>ADL0138</i>	6	21	56	<i>GCT0004</i>	E50C23	0	40
<i>LEI0092</i>	6	28.8	59	<i>ADL0022</i>	Z	0	0
<i>LEI0196</i>	6	67.4	110	<i>MCW0055</i>	Z	28.4	15
<i>ABR0041</i>	7	0	*	<i>MCW0258</i>	Z	38.6	26
<i>MCW0120</i>	7	8.4	44	<i>ADL0273</i>	Z	56.2	73
<i>ABR0397</i>	7	19.8	*	<i>ADL0201</i>	Z	83	87
<i>MCW178</i>	7	33.3	72	<i>MCW0154</i>	Z	97.6	95
<i>ABR0195</i>	7	36.2	*	<i>LEI0121</i>	Z	120	131
<i>MCW0183</i>	7	42.8	86	<i>ABR0119</i>	Z	134.7	*
<i>LEI0158</i>	7	76.4	122	<i>ABR0289</i>	Z	148.3	*
<i>ADL0169</i>	7	105	165				

¹ Kosambi genetic distance from the first marker on each chromosome or linkage group.

² Reference position shown in ArkDB database by Roslin Bioinformatics Group (<http://www.thearkdb.org/>).

*Not located on the consensus linkage map in 2005 (Schmid *et al.*, 2005).

Shamo is one of the most famous Japanese indigenous chicken breeds and it is kept for cock fighting (Tsudzuki, 2003). In contrast, the White Leghorn which originated from Italy has been selected intensely for egg production. Since these breeds have large genetic divergence (Osman *et al.*, 2006), many unique QTLs will be found. This study therefore aims to detect QTLs affecting external egg quality and illustrate age-related changes of the QTLs detected.

Materials and Methods

Animals

An Oh-Shamo male was mated to three White Leghorn females to produce 23 F₁ individuals. Three hundred and eighty-eight F₂ females were created by full-sib matings between four F₁ males and 19 F₁ females. All individuals were reared as described by Tsudzuki *et al.* (2007). Hens were kept under a photoperiod condition of 14 h light and 10 h dark. All the chickens were treated according to the rules described in Standards Relating to the Care and Management of Experimental Animals (Prime Ministers's Office, Japan, 1980) and Guide for the Use of Experimental Animals in Universities (The Ministry of Educations, Science, Sports, and Culture, Japan, 1987).

Phenotypic Measurements

External egg traits were measured at three different production stages: first egg stage, 300 d of hen age, and 400 d of hen age. First 10 eggs laid by hens were measured as data for the first egg stage. Eggs at 300 d and 400 d of ages were collected within two weeks from 300 d and 400 d of age onwards. External egg traits measured were egg weight (EW), eggshell weight (SW), lengths of the long and short axes of

the egg (LLE and LSE), eggshell thickness of the narrow end, blunt end, and equator of the egg (STN, STB, and STE), eggshell strength (SS), and lightness, redness, and yellowness of the eggshell color (SCL, SCR, and SCY). SS was measured at the pole by using an eggshell strength tester (Fujihira Industry, Japan). Eggshell color was measured with a Minolta Chroma Meter CR-300 (Konica Minolta, Japan). In the present study, the first egg stage, 300 d of age, and 400 d of age were referred to as the early (E-), middle (M-), and late (L-) production stages, respectively.

Genotyping and Map Construction

Genomic DNA was extracted from individual blood samples. Fully informative 143 microsatellite markers on 26 autosomes and the Z chromosome (Table 1) were genotyped following the method of Tadano *et al.* (2007). A linkage map was made using the Map Manager QTX b20 software (Manly *et al.*, 2001), following chicken consensus map 2005 (Schmid *et al.*, 2005) on ArkDB database (<http://www.thearkdb.org/>) and the report of Takahashi *et al.* (2005). Total map distance was estimated to be 2,696 cM by using Kosambi map function, covering approximately 68% of the chicken genome (Schmid *et al.*, 2005).

Statistical Analysis

Before QTL analyses, phenotypic data were corrected for the effects of two environmental factors, date of hatch and F₁ dams, using the JMP software version 5.0.1a (SAS Institute Inc.). Phenotypic distributions were also tested whether or not they fit normal distribution by the Shapiro-Wilk's test. Phenotypes of non-normal distribution were approximated to normal distribution using the Box-Cox transformation. In addition, data for the first egg stage were corrected for the

effect of age at the first eggs to remove the effect of hen ages because age at first egg of F_2 hens ranged from 156 d to 292 d (Goto *et al.*, 2011). In addition, QTL analyses for LLE, LSE, and SW were accomplished using the data corrected by EW. SW was used for the trait corrections of SS, STN, STB, and STE because this parameter was significantly correlated with the eggshell quality ($P < 0.05$). Pearson's correlation coefficients (r) among all phenotypes in each stage were estimated by using JMP.

QTL Analysis

QTL analyses were performed with the R/qtl software (Broman *et al.*, 2003). Single-QTL and two-QTL genome scans were performed by the Haley-Knott regression (Haley and Knott, 1992). Genome-wide significant (5%) and suggestive (10%) thresholds were estimated by 1,000 permutations for the autosomes. In the Z chromosome, the significant thresholds that control the genome-wide false positive rate were estimated as described in the method of Broman *et al.* (2006). For an epistatic interaction between two loci, 5% and 10% genome-wide threshold levels were determined by 1,000 permutations according to the method of Broman and Sen (2009).

The percentage of the phenotypic variance explained by a QTL was computed by R/qtl as described by Broman and Sen (2009). To obtain a total contribution of all QTLs detected for any trait to the phenotypic variance, multiple QTL mapping was performed with the R/qtl software.

Results

Trait Analysis

Table 2 shows mean values of 33 external egg traits in the Oh-Shamo and White Leghorn and their F_1 and F_2 progenies. The values of egg weight (EW and SW) and egg size (LLE and LSE) of the Oh-Shamo were higher than those of the others in the early stage. In the middle and late stages, the values of the Oh-Shamo and F_1 were higher than those of the White Leghorn and F_2 , except for the LLE values of White Leghorn in the middle and late stages. The SS value of F_1 was constant at approximately 4 kg/cm² in all stages. In contrast, the SS values of the White Leghorn and F_2 decreased with age, and that of the Oh-Shamo increased. For eggshell colors (SCL, SCR, and SCY), the eggshells of the Oh-Shamo showed darker color than those of the White Leghorn. The values of F_1 showed mid-parental values in all stages. The values of eggshell thickness (STN, STB, and STE) of the Oh-Shamo and F_1 were higher than those of the White Leghorn and F_2 in all stages, except for the STB and STE values of the White Leghorn in the early stage.

The egg weight and size (EW, LLE, and LSE) of the F_2 significantly increased with hens' age ($F_{2,210} = 494.710$, $F_{2,210} = 155.775$, and $F_{2,209} = 588.146$, respectively, $P < 0.0001$ for all traits, one-way repeated measures ANOVA). The SW also significantly increased with age ($F_{2,192} = 116.357$, $P < 0.0001$). Eggshell thickness (STB and STE) remained unchanged throughout the three stages ($F_{2,192} = 2.180$ and $F_{2,192} = 2.649$, respectively, $P > 0.05$), although STN in the middle stage was significantly higher than the other stages ($F_{2,192} =$

11.858, $P < 0.0001$). On the other hand, SS significantly decreased in inverse proportion to increasing age ($F_{2,198} = 79.047$, $P < 0.0001$). Eggshell color (SCL, SCR, and SCY) also became lighter and paler with advancing age ($F_{2,218} = 87.111$, $F_{2,218} = 102.407$, and $F_{2,218} = 102.740$, respectively, $P < 0.0001$ for all traits).

Phenotypic correlations of external egg traits in the F_2 hens are shown in Tables 3–5. Here, traits are confined to six categories, egg weight (EW), eggshell weight (SW), eggshell strength (SS), sizes (LLE and LSE), eggshell thickness (STN, STE, and STB), and colors (SCL, SCR, and SCY) in each stage. Within the same categories, phenotypic correlations between LLE and LSE ranged from 0.60 to 0.73. Phenotypic correlations varied also among STN, STE, and STB (0.67–0.78). On the other hand, phenotypic correlations of SCL vs SCR, and SCL vs SCY were regularly around -0.90 , whereas those of SCR vs SCY were uniformly around 0.90. Among the different categories, phenotypic correlations of EW vs SW, EW vs size, and SW vs size were 0.70–0.79, 0.82–0.96, and 0.49–0.79, respectively. Phenotypic correlations of SW vs thickness, SW vs SS, and SS vs thickness were 0.54–0.75, 0.39–0.45, and 0.40–0.76, respectively. Almost all color traits had no significant phenotypic correlations to EW, SW, SS, size, and thickness.

QTL Analysis

Main-effect QTL: In the present study, no epistatic QTL was discovered for all external egg traits examined. QTLs with main effects on external egg traits were identified on chromosomes 1, 2, 4, 5, 8, 10, 11, 12, 17, and Z as shown in Table 6. For egg weight (E-EW, M-EW, and L-EW), significant QTLs were detected around *MCW0095* (26 cM) on chromosome 8. Between *MCW0240* (201 cM) and *ABR0622* (234 cM) on chromosome 4, a suggestive QTL for E-EW was identified. Each of these QTLs explained 3.94–11.75% of the phenotypic variances.

For size (M-LLE, E-LSE, and L-LSE), significant and suggestive QTLs were detected at around *MCW0154* (95 cM) on the Z chromosome. Between *ADL0372* (0 cM) and *ADL0044* (44 cM) on chromosome 12, significant QTLs for E-LLE and E-LSE were identified. Additionally, a suggestive QTL affecting E-LSE was found between *ADL0272* (44 cM) and *ADL0106* (88 cM) on chromosome 10. Each of these QTLs explained 3.97–5.76% of the phenotypic variances.

For eggshell weight (E-SW, M-SW, and L-SW), significant QTLs were detected around *ABR0119* (135 cM) on the Z chromosome. Around *ABR0530* (0 cM) on chromosome 17, a suggestive QTL for E-SW was identified. Each QTL explained 4.22–7.85% of the phenotypic variances.

For eggshell strength (E-SS and L-SS), significant QTLs were detected between *MCW0258* (26 cM) and *ADL0273* (73 cM) and around *ADL0372* (0 cM) on chromosomes Z and 12, respectively. Each QTL contributed to 4.35–7.96% of the phenotypic variances.

For eggshell thickness (M-STN, L-STN, M-STB, L-STB, and M-STE), significant and suggestive QTLs were found around *MCW0305* (15 cM) and *MCW0095* (26 cM) on chromosome 8. Between *ABR0119* (135 cM) and *ABR0289* (148

Table 2. Means and standard deviations for 33 external egg traits in the Oh-Shamo and White Leghorn breeds and their F₁ and F₂ hens

Trait ¹	Group	Mean±SD (No. of hens)		
		Early stage	Middle stage	Late stage
EW (g)	Oh-Shamo	49.8±5.5 ^a (39)	53.8±4.2 ^a (24)	55.0±4.3 ^a (14)
	White Leghorn	41.3±2.8 ^b (23)	47.4±2.3 ^b (20)	48.6±2.2 ^b (17)
	F ₁	43.7±4.0 ^b (62)	52.6±2.7 ^a (56)	54.5±2.8 ^a (51)
	F ₂	42.2±4.9 ^b (387)	47.6±5.1 ^b (322)	49.4±5.1 ^b (225)
LLE (mm)	Oh-Shamo	53.35±2.30 ^a (39)	55.05±1.47 ^a (24)	55.20±1.77 ^{a,b} (14)
	White Leghorn	51.66±1.98 ^b (23)	53.55±1.38 ^{a,b} (20)	54.49±1.50 ^{a,b} (17)
	F ₁	51.54±1.76 ^b (62)	54.25±1.46 ^a (56)	55.29±1.34 ^a (51)
	F ₂	51.15±2.47 ^b (387)	52.44±2.27 ^b (322)	53.66±2.41 ^b (225)
LSE (mm)	Oh-Shamo	40.74±1.61 ^a (39)	41.87±1.34 ^a (24)	42.02±1.82 ^a (14)
	White Leghorn	37.85±0.72 ^c (23)	40.04±0.62 ^b (20)	40.15±0.65 ^b (17)
	F ₁	38.85±1.28 ^b (62)	41.54±0.74 ^a (56)	42.06±0.84 ^a (51)
	F ₂	38.50±1.55 ^{b,c} (387)	40.46±1.64 ^b (321)	40.82±1.59 ^b (225)
SW (g)	Oh-Shamo	4.8±0.5 ^a (35)	5.0±0.6 ^a (25)	5.2±0.5 ^a (15)
	White Leghorn	4.2±0.4 ^{b,c} (7)	4.4±0.3 ^b (20)	4.5±0.3 ^b (20)
	F ₁	4.4±0.3 ^b (52)	5.2±0.3 ^a (58)	5.2±0.3 ^a (52)
	F ₂	4.2±0.5 ^c (371)	4.5±0.5 ^b (311)	4.6±0.5 ^b (209)
SS (kg/cm ²)	Oh-Shamo	3.61±0.49 ^b (35)	3.93±0.73 ^{a,b} (25)	4.16±0.46 ^a (15)
	White Leghorn	3.67±0.42 ^{a,b} (7)	3.53±0.37 ^b (20)	3.41±0.36 ^b (20)
	F ₁	4.10±0.38 ^a (52)	4.16±0.50 ^a (58)	4.04±0.53 ^a (52)
	F ₂	3.97±0.46 ^a (371)	3.81±0.52 ^b (311)	3.49±0.60 ^b (217)
STN (mm)	Oh-Shamo	0.358±0.021 ^a (35)	0.368±0.030 ^a (25)	0.370±0.020 ^a (15)
	White Leghorn	0.322±0.011 ^b (7)	0.336±0.018 ^b (20)	0.331±0.022 ^b (20)
	F ₁	0.355±0.021 ^a (52)	0.369±0.021 ^a (58)	0.362±0.022 ^a (52)
	F ₂	0.334±0.026 ^b (371)	0.343±0.023 ^b (311)	0.337±0.025 ^b (210)
STB (mm)	Oh-Shamo	0.356±0.023 ^a (35)	0.361±0.031 ^a (25)	0.366±0.023 ^a (15)
	White Leghorn	0.327±0.012 ^{b,c} (7)	0.329±0.019 ^b (19)	0.327±0.019 ^b (20)
	F ₁	0.344±0.016 ^{a,b} (52)	0.361±0.024 ^a (58)	0.356±0.024 ^a (52)
	F ₂	0.323±0.024 ^c (371)	0.326±0.026 ^b (311)	0.321±0.029 ^b (210)
STE (mm)	Oh-Shamo	0.347±0.025 ^a (35)	0.351±0.029 ^b (25)	0.351±0.032 ^a (15)
	White Leghorn	0.332±0.018 ^{a,b} (7)	0.329±0.019 ^c (19)	0.327±0.020 ^b (20)
	F ₁	0.356±0.020 ^a (52)	0.374±0.022 ^a (58)	0.361±0.017 ^a (52)
	F ₂	0.334±0.024 ^b (371)	0.338±0.024 ^c (311)	0.334±0.025 ^b (210)
SCL	Oh-Shamo	75.19±3.14 ^c (21)	77.68±4.91 ^d (23)	80.14±4.24 ^d (14)
	White Leghorn	85.98±2.02 ^a (12)	88.00±1.31 ^a (20)	87.28±1.39 ^a (17)
	F ₁	81.05±2.86 ^b (22)	84.04±2.51 ^c (56)	83.78±2.59 ^c (51)
	F ₂	83.98±2.93 ^a (388)	85.27±2.93 ^b (323)	85.09±2.84 ^b (236)
SCR	Oh-Shamo	6.85±2.39 ^a (21)	5.06±3.07 ^a (23)	3.93±2.94 ^a (14)
	White Leghorn	0.20±1.07 ^d (12)	-0.30±0.31 ^d (20)	-0.29±0.37 ^d (17)
	F ₁	3.80±1.62 ^b (22)	2.19±1.20 ^b (56)	2.30±1.26 ^b (51)
	F ₂	1.98±1.52 ^c (388)	1.34±1.39 ^c (322)	1.29±1.33 ^c (236)
SCY	Oh-Shamo	22.10±2.67 ^a (21)	20.06±4.04 ^a (23)	17.54±3.32 ^a (14)
	White Leghorn	8.27±3.20 ^d (12)	5.57±2.10 ^d (20)	5.34±1.86 ^d (17)
	F ₁	16.56±2.25 ^b (22)	13.86±2.96 ^b (56)	14.68±2.78 ^b (51)
	F ₂	12.44±3.33 ^c (388)	10.82±3.73 ^c (322)	10.89±3.84 ^c (236)

¹ Early stage=first 10 eggs, Middle stage=eggs produced during two weeks from 300 days of age, Late stage=eggs produced during two weeks from 400 days of age, EW=egg weight, LLE=length of the long axis of the egg, LSE=length of the short axis of the egg, SW=eggshell weight, SS=eggshell strength, STN=eggshell thickness at the narrow end of the egg, STB=eggshell thickness at the blunt end of the egg, STE=eggshell thickness at the equator of the egg, SCL=lightness of the eggshell color, SCR=redness of the eggshell color, SCY=yellowness of the eggshell color.

^{a-d} Means with the same superscript letter are not significantly different among the groups at $P>0.05$ in each trait within the same stage (one-way ANOVA followed by Tukey's HSD test).

Table 3. Phenotypic correlations of external egg traits at the early stage in F₂ hens

Trait	E-EW	E-LLE	E-LSE	E-SW	E-SS	E-STN	E-STB	E-STE	E-SCL	E-SCR	E-SCY
E-EW											
E-LLE	0.88										
E-LSE	0.96	0.73									
E-SW	0.79	0.60	0.79								
E-SS	0.07 ^{ns}	−0.07 ^{ns}	0.10	0.45							
E-STN	0.18	−0.02 ^{ns}	0.23	0.59	0.60						
E-STB	0.20	−0.01 ^{ns}	0.25	0.63	0.76	0.75					
E-STE	0.26	0.05	0.23	0.75	0.62	0.78	0.78				
E-SCL	0.06 ^{ns}	0.09 ^{ns}	0.05 ^{ns}	−0.09 ^{ns}	−0.19	−0.16	−0.27	−0.19			
E-SCR	−0.07 ^{ns}	−0.13	−0.04 ^{ns}	0.11 ^{ns}	0.20	0.23	0.30	0.23	−0.95		
E-SCY	−0.05 ^{ns}	−0.14 ^{ns}	−0.01 ^{ns}	0.12	0.19	0.24	0.30	0.25	−0.92	0.93	

Trait abbreviations are shown in Materials and Methods.

^{ns} Not significant at $P > 0.05$.

Table 4. Phenotypic correlations of external egg traits at the middle stage in F₂ hens

Trait	M-EW	M-LLE	M-LSE	M-SW	M-SS	M-STN	M-STB	M-STE	M-SCL	M-SCR	M-SCY
M-EW											
M-LLE	0.82										
M-LSE	0.95	0.64									
M-SW	0.74	0.57	0.66								
M-SS	0.12 ^{ns}	0.02 ^{ns}	0.11 ^{ns}	0.42							
M-STN	0.05 ^{ns}	−0.06 ^{ns}	0.03 ^{ns}	0.56	0.43						
M-STB	0.11 ^{ns}	−0.02 ^{ns}	0.09 ^{ns}	0.61	0.61	0.67					
M-STE	0.14	0.02 ^{ns}	0.10 ^{ns}	0.72	0.48	0.73	0.74				
M-SCL	0.02 ^{ns}	0.05 ^{ns}	0.04 ^{ns}	−0.07 ^{ns}	0.01 ^{ns}	−0.11	−0.10 ^{ns}	−0.03 ^{ns}			
M-SCR	−0.09	−0.11	−0.10	0.01 ^{ns}	−0.07 ^{ns}	0.14	0.08	0.04	−0.92		
M-SCY	−0.04 ^{ns}	−0.07 ^{ns}	−0.05 ^{ns}	0.02 ^{ns}	−0.05 ^{ns}	0.11	0.07 ^{ns}	0.01 ^{ns}	−0.88	0.90	

Trait abbreviations are shown in Materials and Methods.

^{ns} Not significant at $P > 0.05$.

Table 5. Phenotypic correlations of external egg traits at the late stage in F₂ hens

Trait	L-EW	L-LLE	L-LSE	L-SW	L-SS	L-STN	L-STB	L-STE	L-SCL	L-SCR	L-SCY
L-EW											
L-LLE	0.82										
L-LSE	0.94	0.60									
L-SW	0.70	0.49	0.64								
L-SS	0.05 ^{ns}	−0.04 ^{ns}	0.04 ^{ns}	0.39							
L-STN	0.01 ^{ns}	−0.15 ^{ns}	0.02 ^{ns}	0.54	0.41						
L-STB	0.04 ^{ns}	−0.14 ^{ns}	0.04 ^{ns}	0.57	0.49	0.70					
L-STE	0.09	−0.05 ^{ns}	0.07 ^{ns}	0.68	0.40	0.74	0.73				
L-SCL	0.08 ^{ns}	0.06 ^{ns}	0.09 ^{ns}	0.02 ^{ns}	0.04 ^{ns}	−0.12 ^{ns}	−0.01 ^{ns}	−0.05 ^{ns}			
L-SCR	−0.09 ^{ns}	−0.09 ^{ns}	−0.09 ^{ns}	0.03 ^{ns}	0.04 ^{ns}	0.22	0.08 ^{ns}	0.12 ^{ns}	−0.91		
L-SCY	−0.03 ^{ns}	−0.02 ^{ns}	−0.06 ^{ns}	0.05 ^{ns}	0.05 ^{ns}	0.16	0.05 ^{ns}	0.07 ^{ns}	−0.89	0.90	

Trait abbreviations are shown in Materials and Methods.

^{ns} Not significant at $P > 0.05$.

Table 6. Main-effect QTLs affecting external egg traits

Trait ¹	Stage ²	Chr.	Position ³	Flanking markers	CI ⁴	LOD ⁵	% Var. ⁶	Add. ⁷	Dom. ⁸	Differences ⁹	Total contribution ¹⁰
EW	E	4	213	MCW0240-ABR0622	154-250	3.38 [†]	3.94	0.25	0.11	S>H>W	
	E	8	40	MCW0095-ADL0345	29-49	6.70*	7.66	-0.19	0.37	H≥W>S	12.18
	M	8	39	MCW0095-ADL0345	30-47	8.74*	11.75	-0.27	0.48	H≥W>S	11.75
	L	8	35	MCW0305-MCW0095	13-47	5.05*	9.82	-0.24	0.46	H≥W>S	9.82
LLE	E	12	13	ADL0372-ADL0044	0-38	4.37*	5.07	-0.19	0.01	W>H>S	5.07
	M	Z	100	MCW0154-LEI0121	80-118	3.29*	4.60	0.12	—	S>W	4.60
LSE	E	10	4	ADL0272-ADL0106	0-38	3.46 [†]	4.03	0.08	0.03	S≥H>W	
	E	12	24	ADL0372-ADL0044	0-49	4.99*	5.76	0.14	0.03	S>H>W	
	E	Z	85	ADL0201-MCW0154	75-106	3.40*	3.97	-0.05	—	W>S	13.30
	L	Z	104	MCW0154-LEI0121	0-127	2.71 [†]	5.39	-0.08	—	W>S	5.39
SW	E	17	0	ABR0530	0-23	3.37 [†]	4.22	-0.16	0.06	W≥H>S	
	E	Z	144	ABR0119-ABR0289	118-148	6.39*	7.85	0.16	—	S>W	10.60
	M	Z	130	LEI0121-ABR0119	112-148	5.46*	7.77	0.18	—	S>W	7.77
	L	Z	148	ABR0119-ABR0289	113-148	3.62*	7.85	0.18	—	S>W	7.85
SS	E	Z	51	MCW0258-ADL0273	34-77	3.49*	4.35	0.17	—	S>W	4.35
	L	12	0	ADL0372	0-21	3.77*	7.96	-0.27	-0.32	W>S≥H	7.96
STN	E	5	3	MCW0038-MCW0214	0-21	3.29 [†]	4.00	0.15	0.23	H≥S>W	
	E	17	18	ADL0293-MCW0330	6-34	3.74*	4.53	-0.15	-0.23	W>S≥H	
	E	Z	146	ABR0119-ABR0289	36-148	3.28*	3.99	0.15	—	S>W	10.50
	M	8	41	MCW0095-ADL0345	31-70	3.52 [†]	5.08	0.13	-0.30	S>W≥H	5.08
STB	L	8	44	MCW0095-ADL0345	32-55	4.20*	8.85	0.08	-0.56	S>W>H	8.85
	E	Z	144	ABR0119-ABR0289	115-148	3.09*	3.09	0.17	—	S>W	3.09
	M	8	35	MCW0305-MCW0095	26-52	6.13*	8.68	0.27	-0.23	S>H≥W	
	M	Z	141	ABR0119-ABR0289	118-148	3.23*	4.67	0.15	—	S>W	13.10
STE	L	8	15	ABR0017-MCW0305	0-55	3.50 [†]	7.41	0.21	-0.41	S>W≥H	7.41
	E	17	8	ABR0530-ADL0293	0-30	3.90*	4.85	-0.21	-0.08	W>H≥S	
	E	Z	141	ABR0119-ABR0289	118-148	5.90*	7.25	0.17	—	S>W	10.90
	M	8	20	ABR0017-MCW0305	2-44	3.81*	5.49	0.25	-0.04	S>H>W	5.49
SCL	M	5	71	MCW0223-ADL0233	57-83	4.59*	6.33	-0.33	-0.09	W>H>S	6.33
	L	5	73	MCW0223-ADL0233	62-84	5.86*	10.81	-0.42	0.02	W>H>S	10.81
SCR	E	1	138	MCW0112-MCW0058	114-155	4.31*	4.99	0.29	0.07	S>H>W	
	E	4	186	ABR0331-MCW0240	146-255	3.80*	4.42	0.24	0.23	S≥H>W	
	E	5	69	MCW0223-ADL0233	45-85	3.61 [†]	4.20	0.26	0.14	S≥H>W	13.36
	M	1	146	MCW0112-MCW0058	123-200	4.12*	5.73	0.32	-0.12	S>H>W	
SCY	M	5	71	MCW0223-ADL0233	52-86	4.19*	5.81	0.32	0.10	S>H>W	11.69
	L	5	69	MCW0223-ADL0233	50-80	6.07*	11.17	0.44	-0.17	S>H>W	11.17
	E	1	137	MCW0112-MCW0058	119-223	3.95*	4.58	0.26	0.11	S≥H>W	
	E	4	183	ABR0331-MCW0240	147-261	3.82*	4.43	0.22	0.23	S≥H>W	
	E	5	73	MCW0223-ADL0233	60-84	5.72*	6.56	0.31	0.11	S>H>W	15.28
	M	1	150	MCW0112-MCW0058	133-205	4.27*	5.92	0.32	-0.06	S>H>W	
	M	2	0	LEI0124	0-52	3.34 [†]	4.67	0.24	0.17	S≥H>W	
	M	5	72	MCW0223-ADL0233	60-84	5.29*	7.29	0.35	0.10	S>H>W	
	M	11	34	ADL0210-MCW0066	20-49	3.97*	5.53	-0.27	-0.15	W>H≥S	21.34
	L	5	73	MCW0223-ADL0233	64-83	8.56*	15.39	0.52	-0.09	S>H>W	15.39

¹EW=egg weight, LLE=length of the long axis of the egg, LSE=length of the short axis of the egg, SW=eggshell weight, STN=eggshell thickness at the narrow end of the egg, STE=eggshell thickness at the equator of the egg, STB=eggshell thickness at the blunt end of the egg, SCL=lightness of the eggshell color, SCR=redness of the eggshell color, SCY=yellowness of the eggshell color.

²E=early stage (first 10 eggs), M=middle stage (during two weeks from 300 days of age), L=late stage (during two weeks from 400 days of age).

³Chromosomal position (cM) in the present study.

⁴1.5-LOD drop confidence interval in cM.

⁵Genome-wide significant and suggestive QTLs detected (*=5% level, [†]=10% level).

⁶Percentage of the phenotypic variance explained by the QTL.

⁷Additive effect of the QTL shown in standard deviation unit. Positive and negative signs show that the Oh-Shamo (S) allele increased and decreased phenotypic values, respectively.

⁸Dominance effect of the QTL shown in standard deviation unit.

⁹Phenotypic differences among three possible genotypes at the nearest marker locus, two homozygotes for either the Oh-Shamo (S) or White Leghorn (W) allele and heterozygote (H), estimated by one-way ANOVA (followed by Tukey's HSD test).

¹⁰A total contribution of all QTLs detected for the trait to the phenotypic variance, shown in %.

Table 7. Summary of the positions, target traits, and expression stages of the QTLs detected in the present study

QTL no.	Chr.	Markers (position, cM) ¹	Trait categories ²	Traits ²	Stage of QTL expression ³			Phenotypic correlations between stages		
					E	M	L	E-M	E-L	M-L
QTL1	1	<i>MCW0112</i> (205) - <i>MCW0058</i> (241)	eggshell color	SCR	X	X		0.85	0.77	0.87
				SCY	X	X		0.85	0.79	0.86
QTL2	2	Around <i>LEI0124</i> (0*)	eggshell color	SCY		X [†]		0.85	0.79	0.86
QTL3	4	Around <i>MCW0240</i> (201)	egg weight	EW	X [†]			0.74	0.62	0.88
				SCR	X			0.85	0.77	0.87
				SCY	X			0.85	0.79	0.86
QTL4	5	<i>MCW0038</i> (71) - <i>MCW0214</i> (88)	eggshell thickness	STN	X [†]			0.68	0.43	0.61
QTL5	5	<i>MCW0223</i> (123) - <i>ADL0233</i> (151)	eggshell color	SCL		X	X	0.82	0.78	0.86
				SCR	X	X	X	0.85	0.77	0.87
				SCY	X	X	X	0.85	0.79	0.86
QTL6	8	Around <i>MCW0305</i> (15) and <i>MCW0095</i> (26)	egg weight	EW	X	X	X	0.74	0.62	0.88
				STN		X	X	0.68	0.43	0.61
				STB		X	X	0.57	0.35	0.63
				STE		X		0.62	0.52	0.71
QTL7	10	<i>ADL0272</i> (44) - <i>ADL0106</i> (88)	egg size	LSE	X [†]			0.76	0.66	0.87
QTL8	11	<i>ADL0210</i> (54) - <i>MCW0066</i> (69)	eggshell color	SCY		X		0.85	0.79	0.86
QTL9	12	Around <i>ADL0372</i> (0)	egg size	LLE	X			0.61	0.50	0.82
				LSE	X			0.76	0.66	0.87
QTL10	17	Around <i>ADL0293</i> (26)	eggshell strength	SS			X	0.41	0.31	0.55
				SW	X [†]			0.70	0.54	0.77
				STN	X			0.68	0.43	0.61
				STE	X			0.62	0.52	0.71
QTL11	Z	Around <i>ADL0273</i> (73)	eggshell strength	SS	X			0.41	0.31	0.55
QTL12	Z	Around <i>MCW0154</i> (95)	egg size	LLE		X		0.61	0.50	0.82
				LSE	X		X	0.76	0.66	0.87
QTL13	Z	Around <i>ABR0119</i> (135*)	eggshell weight	SW	X	X	X	0.70	0.54	0.77
				STN	X			0.68	0.43	0.61
				STB	X	X		0.57	0.35	0.63
				STE	X			0.62	0.52	0.71

¹ Markers close to QTL and their reference positions. * indicates approximate position because the marker has no reference map position.

² Traits were classified into six categories, *i.e.* egg weight (EW), egg size (LLE and LSE), eggshell weight (SW), eggshell thickness (STN, STB, and STE), eggshell strength (SS), and eggshell color (SCL, SCR, and SCY). Trait abbreviations are shown in Materials and Methods.

³ X indicates the stage when the QTL was detected. E=early stage (first 10 eggs), M=middle stage (during two weeks from 300 days of age), L=late stage (during two weeks from 400 days of age). [†] indicates the QTL at suggestive level.

cM) on the Z chromosome, significant QTLs influencing E-STN, E-STB, M-STB, and E-STE were discovered. Significant QTLs affecting E-STN and E-STE were identified at around *ADL0293* (26 cM) on chromosome 17. In addition, a suggestive QTL for E-STN was found between *MCW0038* (71 cM) and *MCW0214* (88 cM) on chromosome 5. Each QTL accounted for 3.09–8.85% of the phenotypic variances.

For eggshell color (M-SCL, L-SCL, E-SCR, M-SCR, L-SCR, E-SCY, M-SCY, and L-SCY), significant and suggestive QTLs were detected between *MCW0223* (123 cM) and *ADL0233* (151 cM) on chromosome 5. Significant QTLs for E-SCR, M-SCR, E-SCY, and M-SCY were discovered between *MCW0112* (205 cM) and *MCW0058* (241 cM) on chromosome 1. Significant QTLs influencing E-SCR and E-SCY were detected between *ABR0331* (183 cM) and *MCW0240* (201 cM) on chromosome 4. Moreover, significant and suggestive QTLs for M-SCY were found between *ADL0210* (54 cM) and *MCW0066* (69 cM) and around *LEI0124* (0 cM)

on chromosomes 11 and 2, respectively. Each QTL contributed to 4.20–15.39% of the phenotypic variances.

Multiple QTL mapping estimated a total contribution of all QTLs detected for each of external egg traits to the phenotypic variances. This contribution varied from 3.09 to 21.34% depending on the traits.

The QTL alleles derived from the Oh-Shamo uniquely increased most external egg trait values (Table 6), as expected from the higher phenotypic values in the Oh-Shamo than in the White Leghorn (Table 2). However, there were some QTL alleles with opposite effects to phenotypic differences between the Oh-Shamo and the White Leghorn, the phenomenon being called transgressive segregation (Rieseberg *et al.*, 1999). For example, at the EW QTL on chromosome 8, the allele derived from the Oh-Shamo decreased the trait value, in spite of the superior phenotypic value of the Oh-Shamo over the White Leghorn. At the SS QTL on the Z chromosome, the Oh-Shamo allele increased the trait value at

the early stage, regardless of its inferior phenotypic value to the White Leghorn.

As summarized in Table 7, we found 13 independent QTL regions. They were one QTL each on chromosome 1 (between *MCW0112* and *MCW0058*), on chromosome 2 (around *LEI0124*), on chromosome 4 (around *MCW0240*), two QTLs on chromosome 5 (between *MCW0038* and *MCW0214* and between *MCW0223* and *ADL0233*), one QTL each on chromosome 8 (around *MCW0305* and *MCW0095*), on chromosome 10 (between *ADL0272* and *ADL0106*), on chromosome 11 (between *ADL0210* and *MCW0066*), on chromosome 12 (around *ADL0372*), on chromosome 17 (around *ADL0293*), and three QTLs on the Z chromosome (around *ADL0273*, *MCW0154*, and *ABR0119*).

Age-related changes of main-effect QTLs: As shown in Table 7, the present study revealed 13 QTL regions affecting six categories of external egg-related traits, *i.e.* eggshell color, egg weight, egg size, eggshell thickness, eggshell strength, and eggshell weight. In each trait for which the responsible QTL was detected, moderate to high phenotypic correlations were observed among stages. Irrespective of the correlations, however, the responsible QTL was not always found through all the three stages.

For eggshell colors, QTL5 (see Table 7) affected SCR and SCY at all stages from the early to late, while QTL1 for SCR and SCY was found at early and middle stages and QTL5 for SCL was at middle and late stages. QTL3 affected SCR and SCY at the early stage only, and QTL2 for SCY and QTL8 for SCY only at the middle stage.

For egg weight, QTL6 was detected at all stages from the early to late, while QTL3 was at the early stage only. For egg size, QTL12 for LSE was found at the early and late stages, and the same QTL for LLE was at the middle stage only. QTL7 for LSE and QTL9 for LLE and LSE were at the early stage only.

For eggshell thickness, QTL13 for STB was discovered at early and middle stages, QTL6 for STN and STB at middle and late stages, QTL4 for STN, QTL10 for STN and STE, and QTL13 for STN and STE were found at the early stage only, and QTL6 for STE was at the middle stage only. For eggshell strength, QTL11 was detected for SS at the early stage only, and QTL9 was at the late stage only. For eggshell weight, QTL13 was exhibited at all stages from the early to late. QTL10 did so at the early stage only.

Discussion

We discovered 13 external egg quality QTLs on chromosomes 1, 2, 4, 5, 8, 10, 11, 12, 17, and Z in the present study. Among them, six QTLs overlapped in their chromosomal positions with the QTLs reported by other research groups. Around QTL3 (see Table 7) on chromosome 4, many researchers have found a QTL that affects egg weight and/or eggshell colors (Tuiskula-Haavisto *et al.*, 2002; Kerje *et al.*, 2003; Sasaki *et al.*, 2004; Schreiweis *et al.*, 2006; Liu *et al.*, 2011). Around QTL6 on chromosome 8, Yao *et al.* (2010) and Liu *et al.* (2011) have reported QTLs for egg weight and eggshell thickness. The position of QTL8 on

chromosome 11 for eggshell color was similar to that of the QTL found by Sasaki *et al.* (2004). Around QTL9 on chromosome 12, which affects eggshell strength, Tuiskula-Haavisto *et al.* (2010) have detected QTLs affecting eggshell weight and eggshell strength. QTL11 and QTL13 on the Z chromosome for weight and strength of the eggshell also coincided with previously reported QTLs (Tuiskula-Haavisto *et al.*, 2002, 2010; Sasaki *et al.*, 2004). Although there were some overlapping results as described above, the present study found seven novel QTLs. Thus, the accumulation of QTL information with several mapping resources will be very important for understanding the genetic basis of external egg traits.

In the present study, the highest LOD peak was detected for QTL6 on chromosome 8 affecting egg weight (LOD = 8.74). QTL6 also affected eggshell thickness. In addition to our result, several QTLs in this chromosomal region have been reported for egg number, egg shape, and eggshell colors (Tuiskula-Haavisto *et al.*, 2002; Hansen *et al.*, 2005; Yao *et al.*, 2010). Therefore, the QTL region on chromosome 8 should be marked as an important region for the regulation of phenotypic variation of egg traits.

Until now, several QTLs have been reported around the 200 cM region on chromosome 4 (reviewed by Abasht *et al.*, 2006; Hu *et al.*, 2007, 2012). We found QTL3 that affects external egg quality traits (egg weight and eggshell colors) at the region on chromosome 4, in spite of no significant phenotypic correlations among those traits. Moreover, at the similar region, QTLs for several kinds of egg quality traits (weight, shape, and strength of the eggshell and weight of albumen and yolk) have been reported by Sasaki *et al.* (2004), Schreiweis *et al.* (2006), Abasht *et al.* (2009), and Dunn *et al.* (2009). In addition to these, at the region on chromosome 4, Tsudzuki *et al.* (2007) have reported a QTL affecting body weight and shank length. Furthermore, many research groups have also mapped QTLs that affect growth and bone traits around the region (Carlborg *et al.*, 2004; Zhou *et al.*, 2006, 2007; Nadaf *et al.*, 2009; Gu *et al.*, 2011). Therefore, the QTL region may have an important role in bone and egg formation through the calcium metabolic pathway. However, a large number of loci for egg and bone formation would still remain unmapped. Further studies will be required to reveal the genetic control of the traits involved in calcium metabolism.

There were phenotypic correlations among egg weights, egg sizes, eggshell weights, eggshell strengths, and eggshell thicknesses in the present study. Such correlations are likely to occur due to the closely linked loci or pleiotropic loci. We therefore expected prior to QTL mapping that our work would be able to reveal some overlapped loci for these multiple traits. As expected, common QTL regions affecting multiple traits, such as egg weights, egg sizes, eggshell strengths, and eggshell thicknesses were discovered on chromosomes 4, 8, 12, 17, and Z. These loci may explain a part of the reason why the phenotypic correlations were observed. On the other hand, there were few phenotypic correlations between eggshell colors (lightness, redness, and

yellowness) and the other external egg traits, similar to the report of Zhang *et al.* (2005). Thus, prior to QTL mapping, we supposed that the loci affecting eggshell colors would not be consistent with those for the other traits. The present QTL mapping study revealed QTLs on chromosomes 1, 2, 5, and 11 that affect eggshell color traits only, which provided partial evidence that eggshell colors and other external egg traits are controlled by distinct genetic modules.

Among the 13 QTLs detected in the present study, some QTLs expressed in all the three stages and the others did in one or two stage(s) only, irrespective of the existence of positive phenotypic correlations among all the three stages in each trait (Table 7). For example, three QTLs (QTL1, QTL3, and QTL5) were found for the eggshell color trait SCR and high stage correlations ($r=0.77-0.87$) were observed. As expected, QTL5 exerted its effect on all stages. However, QTL1 expressed in both early and middle stages and QTL3 did in the early stage only. Although the reason why such differences in QTL expression occurred is unclear from the present study, there is a possibility that the differences in contributions/effects of each QTL led to the discrepancy of the QTL discovery.

We here revealed that egg weight increases and eggshell color becomes lighter and paler with increasing hens' age, consistent with previous studies (Roland, 1979; Ledur *et al.*, 2002; Odabasi *et al.*, 2007). In the present study, it was found that the size and strength of eggs in F₂ hens increased and decreased, respectively, with aging. Roland (1979) and Odabasi *et al.* (2007) supposed that the increase of surface area of the eggshell and the decrease of eggshell thickness are closely related to the increase of egg weight, and consequently the eggs at the late stage would have lower eggshell pigmentation and lower eggshell quality. We observed a similar trend from the results of our phenotypic analyses for the White Leghorn. On the other hand, the eggshell strength in the Oh-Shamo gradually increased with aging unexpectedly, in spite of increasing egg size. This suggests that the Oh-Shamo has some unique alleles at QTLs affecting eggshell quality in the late stage. Although the present study revealed two QTLs affecting eggshell strength, these were not enough to explain the phenomena. However, it is thought that the Oh-Shamo, an untapped chicken breed, might have a large potential to improve eggshell quality at the late stage of egg laying.

QTLs for eggshell colors were found on chromosomes 1, 2, 4, 5, and 11. Among them, QTLs on chromosomes 1, 2, 4, and 11 (QTL1, QTL2, QTL3, and QTL8) were expressed at the early and/or middle stage(s). These loci contributed to 4.42-5.92% of the phenotypic variances. On the other hand, the QTL on chromosome 5 (QTL5) affected lightness at the middle and late stages, and through the early to late stages for redness and yellowness. In other words, the QTL that appeared at the late stage is the chromosome 5 QTL only. Furthermore, it showed relatively large contributions (10.81-15.39%) at the late stage. These results suggest that the contributions of the chromosome 5 QTL may be higher, due to progressively smaller effects on the traits at the late stage

in QTLs on chromosomes 1, 2, 4, and 11. Consequently, the changes in the contributions/effects of these QTLs may lead to eggshell color becoming lighter and paler with advancing hens' age. The present results suggested that the quality of external egg traits is regulated by multiple loci that shift their contributions depending on age. Hence, QTL analyses with data focused on several production stages will have a large potential to provide further insights of phenotypic changes caused by aging.

We found no epistatic QTLs for external egg quality in the present study. However, some epistatic QTLs have been reported in chickens (Carlborg *et al.*, 2003, 2004, 2006; Cheng *et al.*, 2007; Wahlberg *et al.*, 2009; Ankra-Badu *et al.*, 2010; Tuiskula-Haavisto *et al.*, 2010; Goto *et al.*, 2011). These epistatic loci have substantial effect on phenotypic variance (Carlborg and Haley, 2004). Therefore, QTL mapping studies should be performed with the interaction effects between loci in mind.

In conclusion, 13 QTLs for external egg traits were discovered using the Oh-Shamo, a unique indigenous Japanese breed of chickens. The age-related changes of QTLs illustrate one aspect of the complex genetic basis of external egg traits.

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