ANIMAL GENETICS Immunogenetics, Molecular Genetics and Functional Genomics

doi:10.1111/j.1365-2052.2011.02190.x

Mapping quantitative trait loci for egg production traits in an F_2 intercross of Oh-Shamo and White Leghorn chickens

T. Goto*, A. Ishikawa†, S. Onitsuka*, N. Goto*, Y. Fujikawa*, T. Umino*, M. Nishibori*,‡ and M. Tsudzuki*,‡

*Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima, Hiroshima 739-8528, Japan. †Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya, Aichi 464-8601, Japan. ‡Japanese Avian Bioresource Project Research Center, Hiroshima University, Hiroshima 739-8528, Japan

Summary

We performed quantitative trait locus (QTL) analyses for egg production traits, including age at first egg (AFE) and egg production rates (EPR) measured every 4 weeks from 22 to 62 weeks of hen age, in a population of 421 F_2 hens derived from an intercross between the Oh-Shamo (Japanese Large Game) and White Leghorn breeds of chickens. Simple interval mapping revealed a main-effect QTL for AFE on chromosome 1 and four main-effect QTL for EPR on chromosomes 1 and 11 (three on chromosome 1 and one on chromosome 11) at the genome-wide 5% levels. Among the three EPR QTL on chromosome 1, two were identified at the early stage of egg laying (26–34 weeks of hen age) and the remaining one was discovered at the late stage (54–58 weeks). The alleles at the two EPR QTL derived from the Oh-Shamo breed unexpectedly increased the trait values, irrespective of the Oh-Shamo being inferior to the White Leghorn in the trait. This suggests that the Oh-Shamo, one of the indigenous Japanese breeds, is an untapped resource that is important for further improvement of current elite commercial laying chickens. In addition, six epistatic QTL were identified on chromosomes 2, 4, 7, 8, 17 and 19, where none of the above main-effect QTL were located. This is the first example of detection of epistatic QTL affecting egg production traits. The main and epistatic QTL identified accounted for 4–8% of the phenotypic variance. The total contribution of all QTL detected for each trait to the phenotypic and genetic variances ranged from 4.1% to 16.9% and from 11.5% to 58.5%, respectively.

Keywords chicken, egg production, epistasis, mapping, quantitative trait loci.

Introduction

Until now, chicken breeding programs to improve economically important traits have been performed on the basis of selection for phenotypic values. However, phenotypic values are based on both genetic factors and non-inheritable environmental factors, which often lead to inaccuracy of selection. It is impossible to exclude completely the environmental factors using conventional breeding methods, which result in approximately 70% accuracy of selection (Meuwissen *et al.* 2001; Muir 2007). To increase the accuracy, it will be necessary to perform a direct selection based on genetic information from useful loci expressing economical traits. Such useful loci can be mapped by a quantitative trait locus (QTL) analysis (Weller *et al.* 1988; Haley & Knott 1992). Detected QTL can be used to develop advanced breeding programs such as marker-assisted selection and marker-assisted best linear unbiased prediction (van der Beek & van Arendonk 1996; Totir *et al.* 2004).

In QTL mapping studies, it is necessary to create an F_2 or backcross resource population, and it is ideal that parental animal breeds have diverse genetic backgrounds (Hillel 1997). Thus, QTL mapping populations between different subspecies have been often used in mice (Ishikawa *et al.* 2007). In chickens, some studies have used the Red Junglefowl, known as a wild ancestor of chickens (Schutz *et al.* 2002, 2004). In the present study, we selected the Oh-Shamo and White Leghorn breeds as paternal and maternal breeds, respectively, because these two breeds show marked physiological and morphological differences

Address for correspondence

M. Tsudzuki, Laboratory of Animal Breeding and Genetics, Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima, Hiroshima 739-8528, Japan.
E-mail: tsudzuki@hiroshima-u.ac.jp

Accepted for publication 1 December 2010

as follows. The Oh-Shamo is an indigenous Japanese breed that was developed for cock fighting, with a large erect body and low egg production rate, whereas the White Leghorn is a well-known egg-layer originally from Italy (Roberts 1997; Tsudzuki 2003). Furthermore, Osman *et al.* (2006) revealed, using a microsatellite profiling technique, that the genetic divergence between the two breeds was high (D_A = 0.568–0.575).

At present, over 2200 QTL have been reported to be associated with growth, egg production, meat production, disease resistance, behaviour, and some other traits in chickens (e.g., Abasht *et al.* 2006; Burt 2007; Hu *et al.* 2007). Most of these QTL have significant effects on these traits. Several studies have revealed QTL with epistatic effects on growth traits (Carlborg *et al.* 2003, 2004, 2006; Wahlberg *et al.* 2009), Marek's disease-related traits (Cheng *et al.* 2007) and body composition traits (Ankra-Badu *et al.* 2010). For egg-related traits, approximately 50 QTL exercising the main effect have been reported, and no epistatic QTL have been discovered (Tuiskula-Haavisto *et al.* 2002, 2004; Kerje *et al.* 2003; Wardecka *et al.* 2003; Sasaki *et al.* 2004; Hansen *et al.* 2005; Honkatukia *et al.* 2005; Schreiweis *et al.* 2006; Wright *et al.* 2006).

In this article, we describe QTL with main and epistatic interaction effects on egg production traits in our unique F_2 resource family from an intercross of the Oh-Shamo and White Leghorn breeds.

Materials and methods

Animals

An Oh-Shamo male was mated with three White Leghorn females to produce F_1 chickens. A total of 421 F_2 hens were obtained from full-sib matings of four F_1 males and 19 F_1 females. These F_2 hens were raised as described by Tsudzuki *et al.* (2007). In addition, 40 Oh-Shamos, 23 White Leghorns and 62 F_1 hens were reared for phenotypic comparisons.

Phenotypic measurements

Age at first egg (AFE) and egg production rate (EPR) were recorded for each F_2 hen. AFE was defined as the day when a hen produced her first egg. The number of eggs from individual hens was recorded every 4 weeks from 22 to 62 weeks of hen age. EPR (%) was obtained by dividing the number of eggs laid in 4 weeks by 28 (days). EPR for every 4-week egg production period was sequentially designated beginning with EPR1 (22–26 weeks of hen age) to EPR10 (58–62 weeks).

Marker typing and linkage map construction

DNA was extracted from blood samples of F_2 family members following the method of Tadano *et al.* (2007). A total of 139 microsatellite markers on 25 autosome/linkage groups and the Z chromosome (Table S1) were amplified by PCR with individual DNA templates. All markers used were fully informative, meaning that the two parental breeds used did not have any common alleles at all markers. PCR products were electrophoresed using an automated DNA sequencer. Fragment analyses were performed with GeneMapper software version 3.5 (Applied Biosystems).

A linkage map was constructed using the Kosambi map function of the Map Manager QTX b20 software (Manly *et al.* 2001) by reference to the gene orders in the chicken consensus map 2005 (Schmid *et al.* 2005) from ArkDB of the Roslin Bioinformatics Group (http://www.thearkdb.org/). The total linkage map length constructed in our F_2 population was estimated to be 2650 cM, covering approximately 67% of the chicken genome (Schmid *et al.* 2005).

Statistical analysis

For phenotypic comparisons among Oh-Shamo, White Leghorn, F_1 and F_2 animals, one-way analysis of variance (one-way ANOVA) followed by Tukey's HDS test was carried out with the JMP software version 5.0.1a (SAS Institute Inc.). Phenotypic correlations among all egg production traits in the F_2 hens were calculated using JMP.

Before QTL analyses, phenotypic data were corrected for the effects of two environmental factors, the time at hatch, and F_1 dams, using JMP. QTL analysis was carried out with Map Manager QTX. Simple interval mapping was performed to detect QTL with main effects on egg production traits. Genotypes for QTL on autosomes and the Z chromosome were differently segregating in the F_2 hens. That is, there were two parental types of homozygotes and one type of heterozygote for the autosomes, whereas for the Z chromosome there was only one type of homozygote and one type of heterozygote. Hence, QTL analyses on the autosomes and Z chromosome were performed using the intercross and backcross models of Map Manager QTX, respectively. The estimated likelihood ratio statistics obtained were converted into LOD score by dividing it by 4.605. Genome-wide 5% significant thresholds were estimated by 1000 permutations.

The epistatic interaction effect of two QTL was investigated using the interaction command of Map Manager QTX for all possible pairs of the marker loci used, based on the assumption that a QTL is right at a marker locus. Taking multiple testing into consideration, two significance tests for detection of epistatic QTL were performed as described in Ishikawa *et al.* (2005). Briefly, in the first test, significance of the overall effect was established by 1000 permutations of Map Manager QTX. In the second test, if the overall effect exceeded the genome-wide 5% significance thresholds, then the interaction effect was tested using approximate genome-wide thresholds that were converted from thresholds obtained by the permutation test for the simple interval

mapping described above (two degrees of freedom) into thresholds for the interaction term (four degrees of freedom).

Results

Table 1 presents mean values for egg production traits in the Oh-Shamo and White Leghorn, and their F_1 and F_2 progeny. The mean AFE value for the Oh-Shamo breed was higher than that of the White Leghorn breed. The F_1 and F_2 values were nearly the same as that of the White Leghorn. Apart from the results for statistical comparisons, the F_1 values for EPRs 1–4 were all superior to those of the two parental breeds, clearly showing the presence of heterosis for these four EPRs recorded at the early stage of egg laying. This tendency remained in the F_2. For EPRs 5–7 at the middle stage, the F_1 and F_2 values showed a nearly mid-parental value. At the later stage, the F_1 and F_2 values for EPRs 8–10 were nearly the same value as those of the White Leghorn and Oh-Shamo, respectively.

Table 2 presents phenotypic correlations among egg production traits in the F_2 birds. Correlations between AFE and each EPR were all negative in value. There were no significant correlations of AFE with EPRs in the late stages (from EPR6 to EPR9), whereas significant correlations were seen between AFE and EPRs in the early stages (from EPR1 to EPR5). In addition, no significant correlations were observed between early EPRs (EPR1 and EPR2) and late EPRs (from EPR6 to EPR10).

As shown in Table 3 and Fig. 1, significant main-effect QTL were detected for AFE, EPR2, EPR3 and EPR9. Genome-wide 5% levels for simple interval mapping calculated by permutations were 3.6–3.8 in LOD score for these traits. For AFE, EPR2 and EPR9, only one QTL was found on chromosome 1 (LOD = 7.0, 6.0 and 3.8, respectively). In contrast, two QTL were identified on chromosomes 1 and 11 for EPR3 (LOD = 4.0 and 3.7, respectively). The EPR2 and EPR3 QTL on chromosome 1 were mapped very close to each other, and their alleles that were derived from the Oh-Shamo breed decreased trait values. In contrast, the EPR9 QTL was located in a different chromosomal region [211 cM based on the chicken consensus map (Schmid *et al.* 2005)] from the EPR2 and EPR3 QTL (135 and 129 cM, respectively). The Oh-Shamo allele at the EPR9 QTL increased trait value. The AFE and four EPR QTL that were detected explained 8% and 4–6% of the phenotypic variances, respectively.

In addition to the above main-effect QTL, three pairs of QTL with epistatic interaction effects on AFE, EPR3 and EPR6 [LOD(i) = 5.6, 7.7 and 6.1] were detected on chromosomes 2, 4, 7, 8, 17 and 19 (Table 4). All of these epistatic QTL had no clear main effects on the traits investigated. These loci explained 6–8% of the phenotypic variances, which is comparable to the phenotypic contributions of the main-effect QTL (Table 3).

Table 1 Means and standard deviations for 11 egg production traits in the Oh-Shamo and White Leghorn breeds and their F_1 and F_2 progeny.

Trait[1]	Group	No. of birds	Mean ± SD
AFE (days)	Oh-Shamo	40	246.3 ± 35.5[a]
	White Leghorn	23	198.7 ± 12.3[b,c]
	F_1	62	185.3 ± 27.4[c]
	F_2	395	204.3 ± 29.2[b]
EPR1 (%)	Oh-Shamo	15	0.0 ± 0.0[a]
	White Leghorn	10	0.0 ± 0.0[a]
	F_1	52	22.4 ± 23.2[b]
	F_2	421	4.1 ± 23.2[a]
EPR2 (%)	Oh-Shamo	15	2.9 ± 9.2[a]
	White Leghorn	10	6.8 ± 12.0[a]
	F_1	52	73.6 ± 26.1[b]
	F_2	415	33.5 ± 33.2[c]
EPR3 (%)	Oh-Shamo	15	19.3 ± 31.2[a]
	White Leghorn	10	40.0 ± 37.0[a,b]
	F_1	52	75.2 ± 21.5[c]
	F_2	414	54.5 ± 31.8[b]
EPR4 (%)	Oh-Shamo	15	45.5 ± 28.2[a]
	White Leghorn	20	65.4 ± 20.5[a,b]
	F_1	52	73.6 ± 16.6[b]
	F_2	414	61.6 ± 27.8[a]
EPR5 (%)	Oh-Shamo	15	51.0 ± 26.5[a]
	White Leghorn	20	65.7 ± 18.4[a]
	F_1	52	59.6 ± 21.8[a]
	F_2	412	61.6 ± 25.5[a]
EPR6 (%)	Oh-Shamo	15	47.9 ± 26.5[a]
	White Leghorn	20	73.0 ± 14.8[b]
	F_1	52	63.1 ± 21.3[a,b]
	F_2	410	57.6 ± 25.4[a]
EPR7 (%)	Oh-Shamo	15	46.0 ± 30.7[a]
	White Leghorn	20	71.6 ± 8.9[b]
	F_1	52	60.0 ± 22.5[a,b]
	F_2	408	52.4 ± 26.4[a]
EPR8 (%)	Oh-Shamo	15	48.8 ± 29.2[a,b]
	White Leghorn	20	73.0 ± 8.5[c]
	F_1	52	61.0 ± 20.1[b,c]
	F_2	404	49.1 ± 25.9[a]
EPR9 (%)	Oh-Shamo	15	44.3 ± 27.2[a,b]
	White Leghorn	20	69.7 ± 8.0[c]
	F_1	52	61.3 ± 20.4[b,c]
	F_2	403	46.4 ± 25.7[a]
EPR10 (%)	Oh-Shamo	15	50.0 ± 22.3[a,b]
	White Leghorn	20	61.6 ± 16.3[a]
	F_1	52	51.7 ± 24.2[a,b]
	F_2	400	45.6 ± 24.5[b]

[1]AFE, age at first egg; EPR1, egg production rate from 22 to 26 weeks of age; EPR2, egg production rate from 26 to 30 weeks of age; EPR3, egg production rate from 30 to 34 weeks of age; EPR4, egg production rate from 34 to 38 weeks of age; EPR5, egg production rate from 38 to 42 weeks of age; EPR6, egg production rate from 42 to 46 weeks of age; EPR7, egg production rate from 46 to 50 weeks of age; EPR8, egg production rate from 50 to 54 weeks of age; EPR9, egg production rate from 54 to 58 weeks of age; EPR10, egg production rate from 58 to 62 weeks of age.

[a–c]Means with the same superscript letter are not significantly different among the groups at $P < 0.05$ for each trait (one-way ANOVA followed by Tukey's HDS test).

 doi: 10.1111/j.1365-2052.2011.02190.x

Table 2 Phenotypic correlations among 11 egg production traits in F_2 birds.

Trait	AFE	EPR1	EPR2	EPR3	EPR4	EPR5	EPR6	EPR7	EPR8	EPR9
AFE										
EPR1	−0.39									
EPR2	−0.75	0.46								
EPR3	−0.78	0.15	0.58							
EPR4	−0.52	0.11	0.22	0.60						
EPR5	−0.29	0.09^{ns}	0.08	0.32	0.62					
EPR6	-0.11^{ns}	0.10^{ns}	0.01^{ns}	0.16	0.41	0.63				
EPR7	-0.02^{ns}	0.05^{ns}	-0.01^{ns}	0.08	0.30	0.46	0.60			
EPR8	-0.01^{ns}	0.02^{ns}	-0.05^{ns}	0.07	0.24	0.38	0.51	0.64		
EPR9	-0.05^{ns}	-0.01^{ns}	-0.01^{ns}	0.11	0.26	0.35	0.43	0.49	0.67	
EPR10	−0.10	0.04^{ns}	0.02^{ns}	0.09	0.22	0.36	0.43	0.45	0.50	0.66

See Table 1 for trait abbreviations.

^{ns}Not significant at $P < 0.05$. All the others were all significant at $P < 0.05$.

Table 3 Summary of significant QTL with main effects on egg production traits.

Trait[1]	Chr.	Position[2]	CI[3]	LOD[4]	Var[5]	Additive[6]	Dominance[7]	d/a[8]	Inheritance[9]	Difference[10]
AFE	1	*ADL0188* − 2 (131 cM)	22	7.0	8	0.32	−0.35	−1.1	Rec, Add	S > W ≥ H
EPR2	1	*ADL0188* + 2 (135 cM)	22	6.0	6	−0.27	0.30	−1.1	Rec, Add	H ≥ W > S
EPR3	1	*ADL0188* − 4 (129 cM)	32	4.0	4	−0.25	0.32	−1.3	Rec, Add	H ≥ W > S
	11	*MCW0066* + 0 (69 cM)	8	3.7	4	0.21	0.28	1.3	Dom, Add	H ≥ S > W
EPR9	1	*MCW0112* + 6 (211 cM)	48	3.8	4	0.29	0.21	0.7	Dom, Add, Rec	S ≥ H > W

[1]See Table 1 for trait abbreviations.

[2]The positive and negative signs indicate that the QTL maps that distance (cM) distal and proximal, respectively, to the nearest marker. The number in parentheses indicates map position from the top of the chromosome in the 2005 consensus map (Schmid *et al.* 2005).

[3]The length of the 1.5-LOD drop support interval in cM.

[4]The maximum LOD score significant at the genome-wide 5% level.

[5]The phenotypic variance (%) explained by the QTL.

[6]The additive effect of the QTL shown in the standard deviation unit. The positive value shows that the QTL allele derived from the Oh-Shamo breed increases the trait value.

[7]The dominance effect of the QTL shown in the standard deviation unit.

[8]The degree of dominance.

[9]The most likely mode of inheritance for the QTL determined by two statistical tests (according to the method of Tsudzuki *et al.* 2007) is shown on the left: Rec, recessive; Add, additive; and Dom, dominant.

[10]The phenotypic difference 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 HDS test).

Figure 2 indicates phenotypic effects of three pairs of the six epistatic QTL. Interestingly, double-homozygotes for the Oh-Shamo allele at the two epistatic AFE QTL had the lowest value among nine possible genotypic combinations. This means that the double-homozygotes produce the first egg at the earliest age, in spite of the parental breed Oh-Shamo producing it significantly later than the White Leghorn (Table 1). For EPR3 and EPR6 traits, double-homozygotes for the White Leghorn allele did not exhibit the highest values among nine possible genotypic combinations, although the White Leghorn was superior to the Oh-Shamo in both EPRs. Instead, individuals that had one or two Oh-Shamo alleles were highest in those EPRs (Fig. 2 and Table 1).

Table 5 presents broad-sense heritabilities in AFE and EPR traits and the total contributions of all detected QTL to the phenotypic and genetic variances in AFE and EPRs. Among the QTL summarized in this table, three main-effect QTL for AFE, EPR2 and EPR3 were found in the region around 130 cM on chromosome 1 (see also Table 3). The other main and epistatic QTL were associated with only one trait (see also Tables 3 & 4). This means that most of the detected loci have age-specific effects on egg production traits. Broad-sense heritability was 27% in AFE, while it ranged from 30.2% to 50.0% in EPRs, with the lowest and the highest values in EPR3 and EPR2, respectively. The total contribution to phenotypic and genetic variances of all QTL detected for AFE was 15.8% and 58.5%, respectively,

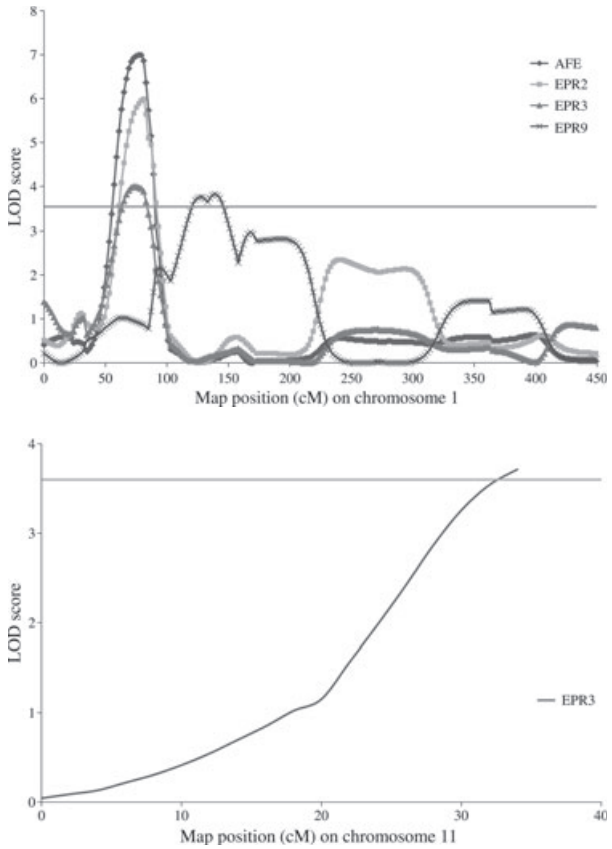

Figure 1 LOD score plots of QTL on chromosomes 1 and 11 with main effects on egg production traits. Upper and lower figures indicate the QTL positions on chromosomes 1 and 11, respectively. Simple interval mapping was performed with the Map Manager QTX b20 software (Manly *et al*. 2001). The horizontal dotted line shows the genome-wide 5% significance level as estimated by 1000 permutations. AFE, age at first egg; EPR2, egg production rate from 26 to 30 weeks of age; EPR3, egg production rate from 30 to 34 weeks of age; EPR9, egg production rate from 54 to 58 weeks of age.

Table 4 Summary of significant QTL with epistatic interaction effects on egg production traits.

Trait[1]	Chr.	QTL 1[2]	Chr.	QTL 2[2]	LOD (t)[3]	LOD (i)[4]	% Var[5]
AFE	7	*LEI0158*	8	*MCW0095*	9.4	5.6	6
EPR3	4	*MCW0240*	17	*ABR0530*	9.4	7.7	8
EPR6	2	*MCW0062*	19	*ABR0180*	10.0	6.1	7

[1]See Table 1 for trait abbreviations.
[2]The marker nearest to the QTL is shown.
[3]LOD score for the total effect.
[4]LOD score for the interaction effect.
[5]The percentage of the phenotypic variance explained by the pair.

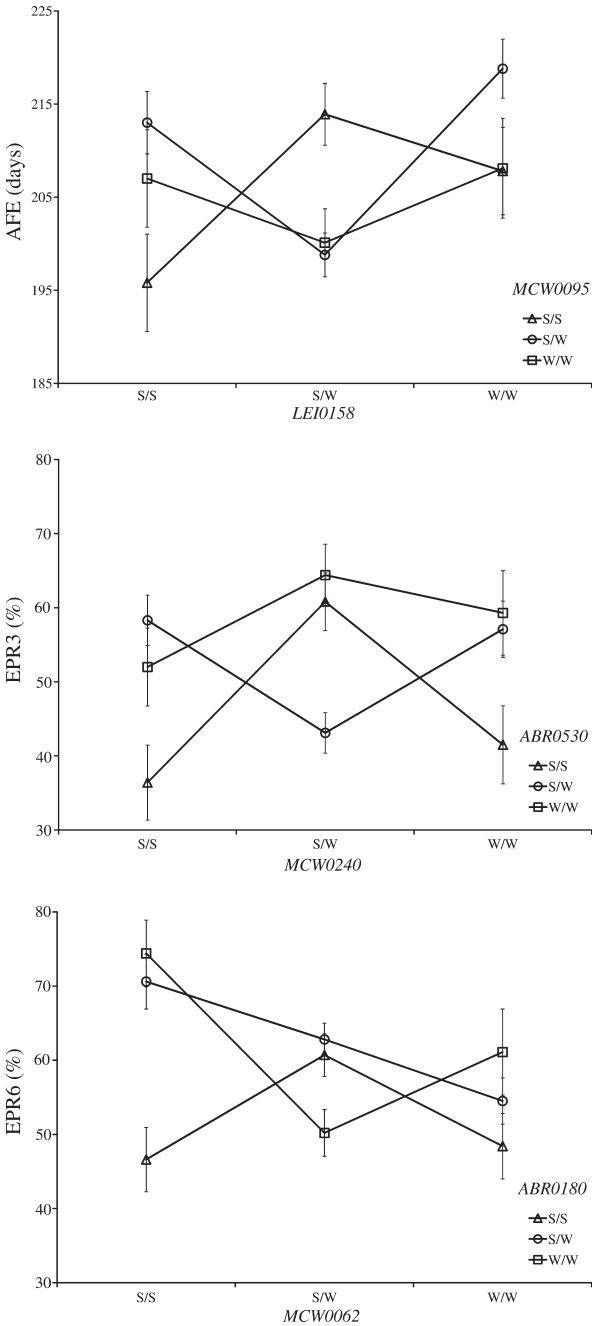

Figure 2 Phenotypic effects of epistatic QTL detected. Upper, middle and lower figures indicate the effects of interactions for the traits of age at first egg (AFE), egg production rate from 30 to 34 weeks of age (EPR3), and egg production rate from 42 to 46 weeks of age (EPR6), respectively. The effects were estimated by two-way ANOVA using the markers nearest the QTL. S and W denote alleles derived from the Oh-Shamo and White Leghorn, respectively.

whereas those QTL detected for EPRs ranged from 4.1% to 16.9% and from 11.5% to 56.1%, respectively. The lowest and highest values for EPRs were obtained in EPR9 and EPR3, respectively.

Discussion

Our phenotypic comparisons among the three-generation families have revealed heterosis for EPRs 1–4 at the early stage of egg laying. The QTL analysis of the F_2 population

Table 5 Total contributions of all QTL detected for egg production traits to the phenotypic and genetic variances.

Trait[1]	Number of main-effect QTL detected	Number of epistatic QTL detected	Total number of QTL detected	Phenotypic variance[2]	Genetic variance[3]	Broad-sense heritability[4]
AFE	1	2	3	15.8	58.5	27.0
EPR2	1	0	1	6.4	12.8	50.0
EPR3	2	2	4	16.9	56.1	30.2
EPR6	0	2	2	6.8	20.8	32.5
EPR9	1	0	1	4.1	11.5	35.4

[1]See Table 1 for trait abbreviations.

[2]The phenotypic variance (%) explained by all detected QTL, which was estimated by a multiple regression analysis with JMP software.

[3]The genetic variance (%) explained by all detected QTL, which was estimated by dividing the phenotypic variance by the broad-sense heritability.

[4]Broad-sense heritabilities were calculated according to the method of Fishman *et al.* (2002).

revealed a single dominant locus affecting EPRs 2 and 3 on chicken chromosome 1. As both loci are not overdominant, unlike the heterotic QTL reported on body weight in the mouse (Ishikawa 2009), from the QTL mapping in this study we cannot explain the causes of heterosis for EPRs 2 and 3.

The two QTL for EPRs 2 and 3 appear to be the same locus, because their map positions, mode of inheritance and phenotypic differences among the three possible genotypes were very similar. However, without further fine-mapping of these loci, we cannot rule out the possibility that they are closely linked.

According to the literature, many QTL influencing egg production, growth, and their related traits are located on chromosome 1. Hansen *et al.* (2005) and Abasht *et al.* (2009) reported QTL for egg production at the early stage of egg laying in a region of 160–205 cM based on the chicken consensus map (Schmid *et al.* 2005); our EPR9 QTL mapped to the 211 cM region on this chromosome. Tuiskula-Haavisto *et al.* (2004) reported a QTL affecting AFE, which is an indicator of the sexual maturity of a hen, in the vicinity of the 205–215 cM region. This locus is relatively distant from our AFE QTL (131 cM). Tsudzuki *et al.* (2007) reported a shank length QTL in the region of 133 cM. Moreover, QTL for growth traits are located in the region at approximately 120–220 cM (Abasht *et al.* 2006; Sharman *et al.* 2007; Atzmon *et al.* 2008; Gao *et al.* 2010). These results suggest that multiple loci with economically important roles for growth, sexual maturity and egg production may be located at 120–220 cM on chromosome 1.

In this study, the Oh-Shamo breed was inferior to the White Leghorn breed in all EPR traits. However, at two QTL affecting EPR3 on chromosome 11 and EPR9 on chromosome 1, the alleles derived from the Oh-Shamo unexpectedly increased the corresponding EPR values. Likewise, Tsudzuki *et al.* (2007) reported that the Oh-Shamo allele at a shank length QTL on chromosome 24 decreased that length, irrespective of the longer shank of the Oh-Shamo compared to the White Leghorn. Thus, unique QTL alleles discovered from the Oh-Shamo, one of the indigenous Japanese chicken breeds, may have great potential for further improvement of egg production traits in elite commercial chicken breeds that have been intensively selected to date.

In this study, we have identified epistatic QTL for AFE on chromosomes 7 and 8, for EPR3 on chromosomes 4 and 17, and for EPR6 on chromosomes 2 and 19. To confirm the presence of these epistatic QTL, we implemented another QTL mapping software, R/qtl, which utilizes a different mapping algorithm to Map Manager QTX (Broman *et al.* 2003). The results obtained from R/qtl were very similar to the results obtained from Map Manager QTX (data not shown). Thus, we believe that the present epistatic effects are genuine. Although several closely located QTL with main effects on egg production have been reported (Tuiskula-Haavisto *et al.* 2002; Hansen *et al.* 2005; Schreiweis *et al.* 2006), to the best of our knowledge no epistatic QTL have been reported. For other traits, Carlborg *et al.* (2004) reported epistatic QTL affecting early growth in a cross between layer and broiler chickens. Thus, this study is the first example of mapping of epistatic loci for egg production traits. In addition, we obtained interesting results showing that the indigenous Japanese breed Oh-Shamo has useful alleles at the epistatic QTL. Therefore, QTL analyses using undeveloped indigenous breeds may lead to the identification of more unique alleles at epistatic QTL as well as QTL with main effects on production traits.

In this study, we identified at least three main QTL and six epistatic QTL affecting egg production traits. Among them, two main QTL and all epistatic QTL displayed age-specific effects on these traits. These results highlight the importance of taking into account the age specificity of QTL when QTL mapping is performed. The percentage of genetic variance explained by these QTL was not so high (11.5–58.5%) for each trait, meaning that many other QTL with small and/or age-specific effects remain unmapped. To map such QTL, more markers and more chickens in the mapping population will be needed.

The candidate genes for our QTL were searched using UCSC Genome Browser (http://genome.ucsc.edu/). A great

number of genes were found in the 1.5-LOD drop support intervals. In the future, the list of candidate genes must be reduced using fine-mapping of the QTL.

In conclusion, several unique QTL with main and epistatic effects on egg production traits were revealed using the untapped Japanese indigenous breed of Oh-Shamo in chickens. This is the first example of the detection of epistatic QTL for this kind of trait.

Acknowledgements

This work was supported in part by a grant-in-aid for Scientific Research (B) (#19380159) from the Ministry of Education, Science, Sports, and Culture, Japan, to M.T. and A.I. We thank Dr L.M. Liao for proofreading the manuscript.

References

Abasht B., Dekkers J.C.M. & Lamont S.J. (2006) Review of quantitative trait loci identified in the chicken. *Poultry Science* **85,** 2079–96.

Abasht B., Sandford E., Arango J. *et al.* (2009) Extent and consistency of linkage disequilibrium and identification of DNA markers for production and egg quality traits in commercial layer chicken populations. *BMC Genomics* **10**(Suppl 2), S2.

Ankra-Badu G.A., Shriner D., Le Bihan-Duval E. *et al.* (2010) Mapping main, epistatic and sex-specific QTL for body composition in a chicken population divergently selected for low or high growth rate. *BMC Genomics* **11,** 107.

Atzmon G., Blum S., Feldman M., Cahaner A., Lavi U. & Hillel J. (2008) QTL detected in a multigenerational resource chicken population. *Journal of Heredity* **99,** 528–38.

van der Beek S. & van Arendonk J.A.M. (1996) Marker-assisted selection in an outbred poultry breeding nucleus. *Animal Science* **62,** 171–80.

Broman K.W., Wu H., Sen S. & Churchill G.A. (2003) R/qtl: QTL mapping in experimental crosses. *Bioinformatics* **19,** 889–90.

Burt D.W. (2007) Emergence of the chicken as a model organism: implications for agriculture and biology. *Poultry Science* **86,** 1460–71.

Carlborg O., Kerje S., Schutz K., Jacobsson L., Jensen P. & Andersson L. (2003) A global search reveals epistatic interaction between QTL for early growth in the chicken. *Genome Research* **13,** 413–21.

Carlborg O., Hocking P.M., Burt D.W. & Haley C.S. (2004) Simultaneous mapping of epistatic QTL in chickens reveals clusters of QTL pairs with similar genetic effects on growth. *Genetical Research* **83,** 197–209.

Carlborg O., Jacobsson L., Ahgren P., Siegel P. & Andersson L. (2006) Epistasis and the release of genetic variation during long-term selection. *Nature Genetics* **38,** 418–20.

Cheng H.H., Zhang Y. & Muir W.M. (2007) Evidence for widespread epistatic interactions influencing Marek's disease virus viremia levels in chicken. *Cytogenetics and Genome Research* **117,** 313–8.

Fishman L., Kelly A.J. & Willis J.H. (2002) Minor quantitative trait loci underlie floral traits associated with mating system divergence in *Mimulus*. *Evolution* **56,** 2138–55.

Gao Y., Du Z.Q., Feng C.G., Deng X.M., Li N., Da Y. & Hu X.X. (2010) Identification of quantitative trait loci for shank length and growth at different development stages in chicken. *Animal Genetics* **41,** 101–4.

Haley C.S. & Knott S.A. (1992) A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity* **69,** 315–24.

Hansen C., Yi N., Zhang Y.M., Xu S., Gavora J. & Cheng H.H. (2005) Identification of QTL for production traits in chickens. *Animal Biotechnology* **16,** 67–79.

Hillel J. (1997) Map-based quantitative trait locus identification. *Poultry Science* **76,** 1115–20.

Honkatukia M., Tuiskula-Haavisto M., de Koning D.J., Virta A., Maki-Tanila A. & Vilkki J. (2005) A region on chicken chromosome 2 affects both egg white thinning and egg weight. *Genetics Selection Evolution* **37,** 563–77.

Hu Z.-L., Fritz E.R. & Reecy J.M. (2007) AnimalQTLdb: a livestock QTL database tool set for positional QTL information mining and beyond. *Nucleic Acids Research* **35,** D604–9.

Ishikawa A. (2009) Mapping an overdominant quantitative trait locus for heterosis of body weight in mice. *The Journal of Heredity* **100,** 501–4.

Ishikawa A., Hatada S., Nagamine Y. & Namikawa T. (2005) Further mapping of quantitative trait loci for post-natal growth in an intersubspecific backcross of wild *Mus musculus castaneus* and C57BL/6J mice. *Genetical Research* **85,** 127–37.

Ishikawa A., Kim E.H., Bolor H., Mollah M.B.R. & Namikawa T. (2007) A growth QTL (*Pbwg1*) region linked loci affecting growth and body composition. *Mammalian Genome* **18,** 229–39.

Kerje S., Carlborg O., Jacobsson L., Schutz K., Hartmann C., Jensen P. & Andersson L. (2003) The twofold difference in adult size between the red junglefowl and White Leghorn chickens is largely explained by a limited number of QTLs. *Animal Genetics* **34,** 264–74.

Manly K.F., Cudmore R.H. Jr & Meer J.M. (2001) Map Manager QTX, cross-platform software for genetic mapping. *Mammalian Genome* **12,** 930–2.

Meuwissen T.H.E., Hayes B.J. & Goddard M.E. (2001) Prediction of genetic value using genome-wide dense marker maps. *Genetics* **157,** 1819–29.

Muir W.M. (2007) Comparison of genomic and traditional BLUP-estimated breeding value accuracy and selection response under alternative trait and genomic parameters. *Journal of Animal Breeding and Genetics* **124,** 342–55.

Osman S.A.M., Sekino M., Nishihata A., Kobayashi Y., Takenaka W., Kinoshita K., Kuwayama T., Nishibori M., Yamamoto Y. & Tsudzuki M. (2006) The genetic variability and relationships of Japanese and foreign chickens assessed by microsatellite DNA profiling. *Asian-Australasian Journal of Animal Sciences* **19,** 1369–78.

Roberts V. (1997) *British Poultry Standards*, 5th edn. pp. 145–50. Blackwell Science, Oxford.

Sasaki O., Odawara S., Takahashi H. *et al.* (2004) Genetic mapping of quantitative trait loci affecting body weight, egg character and egg production in F_2 intercross chickens. *Animal Genetics* **35,** 188–94.

Schmid M., Nanda I., Hoehn H. *et al.* (2005) Second report on chicken genes and chromosomes 2005. *Cytogenetic and Genome Research* **109,** 415–79.

 doi: 10.1111/j.1365-2052.2011.02190.x

Schreiweis M.A., Hester P.Y., Settar P. & Moody D.E. (2006) Identification of quantitative trait loci associated with egg quality, egg production, and body weight in an F_2 resource population of chickens. *Animal Genetics* **37,** 106–12.

Schutz K.E., Kerje S., Carlborg O., Jacobsson L., Andersson L. & Jensen P. (2002) QTL analysis of a red junglefowl × White Leghorn intercross reveals trade-off in resource allocation between behavior and production traits. *Behavior Genetics* **32,** 423–33.

Schutz K.E., Kerje S., Jacobsson L., Forkman B., Carlborg O., Andersson L. & Jensen P. (2004) Major growth QTLs in fowl are related to fearful behavior: possible genetic links between fear responses and production traits in a red junglefowl × White Leghorn intercross. *Behavior Genetics* **34,** 121–30.

Sharman P.W., Morrice D.R., Law A.S., Burt D.W. & Hocking P.M. (2007) Quantitative trait loci for bone traits segregating independently of those for growth in an F_2 broiler x layer cross. *Cytogenetic and Genome Research* **117,** 296–304.

Tadano R., Sekino M., Nishibori M. & Tsudzuki M. (2007) Microsatellite marker analysis for the genetic relationships among Japanese long-tailed chicken breeds. *Poultry Science* **86,** 460–9.

Totir L.R., Fernando R.L., Dekkers J.C., Fernandez S.A. & Guldbrandtsen B. (2004) The effect of using approximate gametic variance covariance matrices on marker assisted selection by BLUP. *Genetics Selection Evolution* **36,** 29–48.

Tsudzuki M. (2003) Japanese native chickens. In: *The Relationship Between Indigenous Animals and Humans in APEC Region* (Ed. by H.L. Chang & Y.C. Huang), pp. 91–116. The Chinese Society of Animal Science, Tainan.

Tsudzuki M., Onitsuka S., Akiyama R., Iwamizu M., Goto N., Nishibori M., Takahashi H. & Ishikawa A. (2007) Identification of quantitative trait loci affecting shank length, body weight and carcass weight from the Japanese cockfighting chicken breed, Oh-Shamo (Japanese Large Game). *Cytogenetic and Genome Research* **117,** 288–95.

Tuiskula-Haavisto M., Honkatukia M., Vilkki J., de Koning D.J., Schulman N.F. & Maki-Tanila A. (2002) Mapping of quantitative trait loci affecting quality and production traits in egg layers. *Poultry Science* **81,** 919–27.

Tuiskula-Haavisto M., de Koning D.J., Honkatukia M., Schulman N.F., Maki-Tanila A. & Vilkki J. (2004) Quantitative trait loci with parent-of-origin effects in chicken. *Genetical Research* **84,** 57–66.

Wahlberg P., Carlborg O., Foglio M., Tordoir X., Syvanen A.C., Lathrop M., Gut I.G., Siegel P.B. & Andersson L. (2009) Genetic analysis of an F_2 intercross between two chicken lines divergently selected for body-weight. *BMC Genomics* **10,** 248.

Wardecka B., Olszewski R., Jaszczak K., Zieba G. & Pierzchala M. (2003) Preliminary mapping of QTLs affecting egg quality on chromosomes 1-5 in chickens. *Czech Journal of Animal Science* **48,** 97–105.

Weller J.I., Soller M. & Brody T. (1988) Linkage analysis of quantitative traits in an interspecific cross of tomato (*Lycopersicon esculentum* × *Lycopersicon pimpine llifolium*) by means of genetic markers. *Genetics* **118,** 329–39.

Wright D., Kerje S., Lundstrom K., Babol J., Schutz K., Jensen P. & Andersson L. (2006) Quantitative trait loci analysis of egg and meat production traits in a red junglefowl × White Leghorn cross. *Animal Genetics* **37,** 529–34.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 The microsatellite markers genotyped in this study.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.