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# Investigating the genetic determination of clutch traits in laying hens

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## Abstract

Clutch traits were proposed as a more detailed description of egg-laying patterns than simple total egg production. In this study, egg production of 23,809 Rhode Island Red (RIR) and 22,210 White Leghorn (WL) hens was described in terms of number of clutches, average and maximum clutch size, age at first egg, total saleable egg production, and percentage of egg defects. Genetic parameters were estimated using a six-trait animal model. Of the phenotyped birds, 1433 RIR hens and 1515 WL hens were genotyped with line specific 50K Affymetrix Axiom single nucleotide polymorphism chips to perform genome-wide association analyses. Moderate heritabilities were estimated for clutch traits of 0.20 to 0.42 in the RIR line and 0.29 to 0.41 in the WL line. Average and maximum clutch size was positively genetically correlated with total saleable egg number in both lines. Genome-wide association analysis identified seven regions that were associated with egg production in the RIR line and 12 regions in the WL line. The regions identified were line and trait specific, except for one region on chromosome 6 from 28 to 29 Mb that influenced number of clutches and maximum and average clutch size in WL hens. Regions associated with egg production identified here overlapped with 260 genes, with some strong positional candidates based on gene ontology including *WASH1*, which is involved in oocyte maturation, *NPVF*, involved in regulation of follicle-stimulating hormone secretion, and *FOXO3*, involved in oocyte maturation and ovulation from the ovarian follicle. Confirmation of the role of these genes in regulation of egg production pattern will require further studies.



## Keywords

clutch trait, GWAS, genetic parameters, layer chicken


## Disciplines

Agriculture | Animal Sciences | Genetics and Genomics | Poultry or Avian Science


## Comments

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# GENETICS AND GENOMICS

## Investigating the genetic determination of clutch traits in laying hens

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**ABSTRACT** Clutch traits were proposed as a more detailed description of egg-laying patterns than simple total egg production. In this study, egg production of 23,809 Rhode Island Red (RIR) and 22,210 White Leghorn (WL) hens was described in terms of number of clutches, average and maximum clutch size, age at first egg, total saleable egg production, and percentage of egg defects. Genetic parameters were estimated using a six-trait animal model. Of the phenotyped birds, 1433 RIR hens and 1515 WL hens were genotyped with line specific 50K Affymetrix Axiom single nucleotide polymorphism chips to perform genome-wide association analyses. Moderate heritabilities were estimated for clutch traits of 0.20 to 0.42 in the RIR line and 0.29 to 0.41 in the WL line. Average and maximum clutch size was positively genetically correlated with total saleable egg number in both lines. Genome-wide association analysis identified seven regions that were associated with egg production in the RIR line and 12 regions in the WL line. The regions identified were line and trait specific, except for one region on chromosome 6 from 28 to 29 Mb that influenced number of clutches and maximum and average clutch size in WL hens. Regions associated with egg production identified here overlapped with 260 genes, with some strong positional candidates based on gene ontology including *WASH1*, which is involved in oocyte maturation, *NPVF*, involved in regulation of follicle-stimulating hormone secretion, and *FOXO3*, involved in oocyte maturation and ovulation from the ovarian follicle. Confirmation of the role of these genes in regulation of egg production pattern will require further studies.

**Key words:** clutch trait, GWAS, genetic parameters, layer chicken

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## INTRODUCTION

The process of egg formation in layers requires on average just over 24 h. As a result, a specific pattern of clutches (number of consecutive days with eggs) and breaks between them occurs. In a natural environment, the hen would cease laying and brood the eggs after a clutch is completed. The invention of artificial incubation removed the need for brooding behavior, which in fact is considered detrimental and is avoided in commercial egg production. Selection for increased egg number favored hens that produce eggs in long clutches with minimum pause lengths between them. Clutch size can be used to describe the individual laying pattern and has been considered as a trait for selection (Noda et al., 2002; Chen and Tixier-Biochard, 2003a,b; Wolc et al., 2010). Evaluation of clutch traits can provide additional insight into the difference between highly productive birds and inferior layers, compared to a simple total egg number count or rate of lay. As a more basic biological trait, clutch pattern may be more heritable and show a stronger association with single nucleotide polymorphisms (**SNP**) than total egg number, particularly considering that the rate of egg lay has been the primary trait for long-term selection in populations destined for production of commercial egg layers. Therefore, it is expected that intense selection has eroded a good proportion of the genetic variability in components associated to rate of lay. The hen's follicle maturation hierarchy and ovulatory cycling are partially controlled by a circadian rhythm that governs the timing of the pre-ovulatory peak of luteinizing hormone, which is influenced by the daily light photoperiod (the length of the light vs. dark cycles). This hormone controls the growth and maturation hierarchy of follicles, thereby marking the ovoposition rhythm. Additional details on the biology of egg formation and its cyclicity have been studied by Lillpers and Wilhelmson (1993) and Luc et al. (1996). Exploring the biological components that control the sequence of ovoposition and its rhythm in more detail could help


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identify components of the egg production complex that may not be subject to direct selection and that could be used as candidates for genetic improvement.

The objective of this study was to estimate genetic parameters of traits that define the clutch pattern in two layer lines from two different breeds and to identify genomic regions associated with clutch length and number.

## MATERIAL AND METHODS

### Phenotypes

Data were obtained from two commercial egg production lines, one is of the Rhode Island Red (**RIR**) breed (brown egg shell) and the other is of the White Leghorn (**WL**) breed for white egg shell production. These lines have been under intensive selection for multiple generations for numerous traits of relevance for commercial egg production, including internal and external egg quality traits, body size, feed efficiency, behavior traits, and especially for egg production and disease resistance. The lines were kept on separate farms and their hatch dates and life event schedules were different, thus direct comparison of the lines was not an objective of this study. In order to characterize laying patterns, daily egg production data from three generations of the RIR (23,809 hens) and WL (22,210 hens) lines were summarized. The pedigree was expanded to include two additional generations of ancestors and consisted of five generations of hens: 101,866 for RIR line and 45,404 for WL line. Days when data recording was disrupted were removed. Within generation records were summarized until the same age for all hatches. Days with two eggs were split into 2 d of one egg if the preceding or subsequent day had no eggs, otherwise both eggs were counted on the same day. All eggs were counted as parts of clutches, including those with defects. Each line was analyzed separately.

A clutch was defined as the number of eggs laid on consecutive days without a break. Starting with the first egg laid by a hen, the following parameters were calculated: number of clutches (**numC**), maximum number of eggs in a clutch (**maxC**), average number of eggs in a clutch (**avgC**), and the average number of eggs in a clutch until 45 wk of age (avgC45wk). To investigate genetic correlations with standard traits used for selection, age at first egg (**afe**), total number of saleable egg (**gEgg**), and percentage of defective eggs (**def**) were also included in the analysis.

### Genotypes

For genome-wide association analysis, available SNP genotypes and phenotypes on 1433 RIR hens and 1515 WL hens were used. Hens were genotyped using line specific 50K Axiom SNP chips (Affymetrix, Santa Clara CA), using plates of 384 samples. Genotype calling was performed by plate, with the following quality criteria (Axiom™, 2017): DishQC > 0.82, which measures the relative intensity of signal to background noise, call rate > 97, Fisher's linear discriminant > 5.25, which is a measure of cluster quality, SNP heterozygous strength offset > –0.1, which measures the offset of the average signal for the heterozygous cluster relative to the average signal for the homozygote clusters in vertical dimension, and homozygote ratio offset > 1.0, which is the location in horizontal dimension of the homozygous genotype cluster center that is closest to heterozygote position. Parentage errors were corrected and missing genotypes were imputed using FImpute (Sargolzaei et al., 2014). Those SNPs with a minor allele frequency below 0.01 were removed from the analysis, which resulted in 52,501 SNPs for the RIR and 41,327 SNPs for the WL line.

### Methods

(Co)variance components were estimated with the following linear multi-trait animal model in ASReml4 (Gilmour et al., 2009), assuming multi-variate normal distributions:

$$\begin{bmatrix} \boldsymbol{y}_1 \\ \boldsymbol{y}_2 \\ \boldsymbol{y}_3 \\ \boldsymbol{y}_4 \\ \boldsymbol{y}_5 \\ \boldsymbol{y}_6 \end{bmatrix} = \begin{bmatrix} \boldsymbol{X}_1 & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{X}_2 & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{X}_3 & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{X}_4 & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{X}_5 & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{X}_6 \end{bmatrix} \begin{bmatrix} \boldsymbol{b}_1 \\ \boldsymbol{b}_2 \\ \boldsymbol{b}_3 \\ \boldsymbol{b}_4 \\ \boldsymbol{b}_5 \\ \boldsymbol{b}_6 \end{bmatrix}$$

$$+ \begin{bmatrix} \boldsymbol{Z}_1 & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{Z}_2 & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{Z}_3 & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{Z}_4 & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{Z}_5 & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{Z}_6 \end{bmatrix} \begin{bmatrix} \boldsymbol{a}_1 \\ \boldsymbol{a}_2 \\ \boldsymbol{a}_3 \\ \boldsymbol{a}_4 \\ \boldsymbol{a}_5 \\ \boldsymbol{a}_6 \end{bmatrix} + \begin{bmatrix} \boldsymbol{e}_1 \\ \boldsymbol{e}_2 \\ \boldsymbol{e}_3 \\ \boldsymbol{e}_4 \\ \boldsymbol{e}_5 \\ \boldsymbol{e}_6 \end{bmatrix}$$

where:

$\boldsymbol{y}_i$ = vector of observations for traits $i$ = afe, avgC, maxC, numC, gEggs, and def

$\boldsymbol{X}_i$ = known design matrix for fixed effects for trait $i$.

$\boldsymbol{b}_i$ = vector of fixed effects of hatch of hen and a covariate for days that the bird was alive during the testing period.

$\boldsymbol{Z}_i$ = known design matrix of random additive genetic effects

$\boldsymbol{a}_i$ = vector of random additive genetic effects

$\boldsymbol{e}_i$ = vector of random errors.

The following covariance structure was assumed:

$$\text{Var}\begin{bmatrix} \boldsymbol{a}_1 \\ \boldsymbol{a}_2 \\ \boldsymbol{a}_3 \\ \boldsymbol{a}_4 \\ \boldsymbol{a}_5 \\ \boldsymbol{a}_6 \end{bmatrix} = \begin{bmatrix} \boldsymbol{\sigma}^2_{a1} & \boldsymbol{\sigma}_{a12} & \boldsymbol{\sigma}_{a13} & \boldsymbol{\sigma}_{a14} & \boldsymbol{\sigma}_{a15} & \boldsymbol{\sigma}_{a16} \\ \boldsymbol{\sigma}_{a21} & \boldsymbol{\sigma}^2_{a2} & \boldsymbol{\sigma}_{a23} & \boldsymbol{\sigma}_{a24} & \boldsymbol{\sigma}_{a25} & \boldsymbol{\sigma}_{a26} \\ \boldsymbol{\sigma}_{a31} & \boldsymbol{\sigma}_{a32} & \boldsymbol{\sigma}^2_{a3} & \boldsymbol{\sigma}_{a34} & \boldsymbol{\sigma}_{a35} & \boldsymbol{\sigma}_{a36} \\ \boldsymbol{\sigma}_{a41} & \boldsymbol{\sigma}_{a42} & \boldsymbol{\sigma}_{a43} & \boldsymbol{\sigma}^2_{a4} & \boldsymbol{\sigma}_{a45} & \boldsymbol{\sigma}_{a46} \\ \boldsymbol{\sigma}_{a51} & \boldsymbol{\sigma}_{a52} & \boldsymbol{\sigma}_{a53} & \boldsymbol{\sigma}_{a54} & \boldsymbol{\sigma}^2_{a5} & \boldsymbol{\sigma}_{a56} \\ \boldsymbol{\sigma}_{a61} & \boldsymbol{\sigma}_{a62} & \boldsymbol{\sigma}_{a63} & \boldsymbol{\sigma}_{a64} & \boldsymbol{\sigma}_{a65} & \boldsymbol{\sigma}^2_{a6} \end{bmatrix} \otimes \boldsymbol{A},$$

$$\text{Var}\begin{bmatrix} e_1 \\ e_2 \\ e_3 \\ e_4 \\ e_5 \\ e_6 \end{bmatrix} = \begin{bmatrix} \sigma^2_{e1} & \sigma_{e12} & \sigma_{e13} & \sigma_{e14} & \sigma_{e15} & \sigma_{e16} \\ \sigma_{e21} & \sigma^2_{e2} & \sigma_{e23} & \sigma_{e24} & \sigma_{e25} & \sigma_{e26} \\ \sigma_{e31} & \sigma_{e32} & \sigma^2_{e3} & \sigma_{e34} & \sigma_{e35} & \sigma_{e36} \\ \sigma_{e41} & \sigma_{e42} & \sigma_{e43} & \sigma^2_{e4} & \sigma_{e45} & \sigma_{e46} \\ \sigma_{e51} & \sigma_{e52} & \sigma_{e53} & \sigma_{e54} & \sigma^2_{e5} & \sigma_{e56} \\ \sigma_{e61} & \sigma_{e62} & \sigma_{e63} & \sigma_{e64} & \sigma_{e65} & \sigma^2_{e6} \end{bmatrix} \otimes \boldsymbol{I}$$

Where $\boldsymbol{\sigma_{aij}}$ is the additive genetic covariance between traits $i$ and $j$, $\boldsymbol{A}$ is the additive genetic relationship matrix based on pedigree, $\boldsymbol{\sigma_{eij}}$ is the residual covariance between traits $i$ and $j$, and $\boldsymbol{I}$ is the identity matrix.

Relative selection efficiency for saleable egg number from selection on clutch traits was calculated using the following formula (Searle, 1978):

$$p = r\sqrt{\frac{h^2_{clutch}}{h^2_{gEgg}}},$$

where r is the genetic correlation between the clutch trait and saleable egg number and $h^2_{clutch}$ and $h^2_{gEgg}$ are respective heritabilities.

Genome-wide association analyses were performed using GenSel4 (Garrick and Fernando, 2013). The model included an overall mean, hatch within generation, a covariate for days that the bird was alive during the testing period, and random SNP effects. Method BayesB of Meuwissen et al. (2001) was applied, a priori fitting 2% of SNPs for the RIR and 3% for the WL line in each of 65,000 iterations, with the first 5000 iterations discarded as burn-in. The number of SNPs fitted per iteration was chosen so as not to exceed the number of genotyped individuals with phenotype in the analysis. The genome was divided into 1 Mb windows based on Galgal4, resulting in 964 windows for the RIR and 973 windows for the WL line, and the proportion of genetic variance explained by each window was calculated. Windows explaining more than 1% of genetic variance and having at least one SNP with non-zero effect in over 90% of iterations (window posterior proportion of inclusion $> 0.9$) were considered to be associated with the analyzed trait. Genomic regions associated with the analyzed traits were overlaid with quantitative trait loci (**QTL**) for egg production traits form the www.animalgenome.org database. Genes included within associated 1 Mb regions and their GO terms were identified using BioMart (http://jul2016.archive.ensembl.org/biomart/martview/48a5cf317f0f0cb22f08f3219a9891d4) and tested for overrepresentation of gene ontology terms using Panther (http://www.pantherdb.org/) with Bonferroni correction.

## RESULTS AND DISCUSSION

Description of egg production traits in the two analyzed lines is in Table 1. There were important differences between the lines in all analyzed traits. The RIR hens were more prolific, with on average 5 more saleable eggs than the WL hens. The number of days alive was only to a small extent affected by mortality and was mainly a management decision due to farm schedules in different years. It is important to clarify that these two lines are kept separated, and that their hatch dates and life event schedules, even though similar, can vary each generation and are, therefore, subject to generation-specific effects due to differences in weather, management, feed, farm personnel, etc. The White egg layers matured on average 3 d earlier and had slightly lower proportion of defective eggs than brown egg layers. The RIR line had longer clutches both in terms of average (15.3) and maximal clutch size (81.8) than the WL line (8.6 and 57.3). The longest clutch recorded was 377 eggs in RIR and 296 in WL. To obtain records more comparable to other publications, the average clutch size was also calculated for the early lay period (until 45 wk of life), during which the clutch length was on average longer than over the whole production cycle, by 10.6 eggs in the RIR line and 8.2 eggs in the WL line, showing a tendency for shorter clutches at older age. A similar tendency towards shortening clutches with age was found by Bednarczyk et al. (2000).

Literature results show average clutch sizes of 8.3, 4.6, and 5.9 at 31 to 51 wk for three lines selected for egg number, egg mass, and feed consumption, respectively (Lillpers and Wilhelmson, 1993). In a selection experiment by Chen and Tixier-Boichard (2003b), 16 generations of selection for average clutch length up to 42 wk resulted in an increase from 4 to 15 eggs per clutch. In a WL population selected for egg production for 29 generations, clutch traits up to 40 wk averaged 11.1 d, 12.7, 38.2 d, 23.7 d, and 2.2 d for clutch length, number of clutches, maximum clutch length, total pause days, and interval between clutches, respectively (Roy et al., 2014). In a commercial WL line, exact ovoposition recording was measured using a transponder nest. The line had on average 11 clutches (maximum 28), with an average length of 10 (maximum 107) days, during a 138-d recording period (Icken et al., 2008). In the WL line used in their experiment, the mean time interval between ovopositions was of 24 h and 6 min +/–37 min.

### *Estimates of Genetic Parameters*

Estimates of genetic parameters for clutch related traits are in Tables 2 and 3. The highest heritability was estimated for age at first egg (0.55 in both lines), as expected. In the literature, age at first egg was reported as a trait with moderate heritability (0.36–0.55; Wolc et al., 2010, 2011; Niknafs et al., 2012; Shad et al., 2013), in agreement with our estimates. Average clutch length and number of clutches had heritabilities similar or higher than total saleable egg production in both lines (0.31–0.42 and 0.34–0.41 vs. 0.26 and 0.34). Using a sire model, Luc et al. (1996) estimated heritabilities of 0.43 and 0.68 for clutch number and 0.49 and 0.50 for average clutch length in two WL lines divergently

**Table 1.** Description of the analyzed traits in the 23,809 RIR and 22,210 WL lines.

| | RIR | | | | WL | | | |
|---|---|---|---|---|---|---|---|---|
| Trait | Mean | STD | Min | Max | Mean | STD | Min | Max |
| daysAlive (d) | 547.5 | 86.0 | 137 | 632 | 574.2 | 91.0 | 134 | 638 |
| afe (d) | 142.7 | 6.8 | 121 | 191 | 140.0 | 7.2 | 112 | 199 |
| numC (pct) | 29.2 | 16.9 | 1 | 118 | 43.4 | 18.0 | 1 | 142 |
| maxC (eggs) | 81.8 | 39.9 | 1 | 377 | 57.3 | 33.5 | 1 | 296 |
| avgC (eggs) | 15.3 | 9.9 | 1 | 252 | 8.6 | 4.4 | 1 | 153 |
| avgC45wk (eggs) | 25.9 | 19.6 | 1 | 187 | 16.8 | 11.4 | 1 | 179 |
| gEgg (eggs) | 319.8 | 83.3 | 1 | 452 | 314.7 | 80.4 | 1 | 434 |
| def (%) | 4.1 | 4.5 | 0 | 95 | 3.5 | 4.1 | 0 | 81 |

daysAlive—number of days that the bird was alive during the egg collection period, afe—age at first egg, numC—number of clutches, maxC—maximum clutch size, avgC—average clutch size, avgC45wk—average clutch size until 45 wk of life, gEgg—total number of saleable eggs, def (%)—percentage of defective eggs.

**Table 2.** Estimates of heritability (on diagonal) and of genetic (above diagonal) and phenotypic correlations (below diagonal) in the RIR line.

| | afe | avgC | maxC | numC | gEgg | def |
|---|---|---|---|---|---|---|
| afe | **0.55 ± 0.02** | –0.07 ± 0.04 | –0.16 ± 0.04 | 0.05 ± 0.03 | –0.24 ± 0.04 | –0.04 ± 0.04 |
| avgC | –0.06 ± 0.01 | **0.31 ± 0.02** | 0.88 ± 0.02 | –0.83 ± 0.01 | 0.57 ± 0.03 | 0.05 ± 0.04 |
| maxC | –0.09 ± 0.01 | 0.58 ± 0.00 | **0.20 ± 0.01** | –0.82 ± 0.02 | 0.61 ± 0.03 | 0.00 ± 0.05 |
| numC | 0.04 ± 0.01 | –0.66 ± 0.00 | –0.53 ± 0.01 | **0.42 ± 0.02** | –0.55 ± 0.01 | –0.11 ± 0.04 |
| gEgg | –0.20 ± 0.01 | 0.44 ± 0.01 | 0.11 ± 0.00 | –0.58 ± 0.03 | **0.26 ± 0.02** | –0.59 ± 0.03 |
| def | 0.01 ± 0.01 | –0.12 ± 0.01 | –0.11 ± 0.01 | 0.12 ± 0.01 | –0.56 ± 0.01 | **0.28 ± 0.02** |
| $h^2_m$ | 0.39 | 0.18 | 0.12 | 0.30 | 0.18 | 0.13 |

Trait names as in Table 1, $h^2_m$—proportion of variance explained by markers.

**Table 3.** Estimates of heritability (on diagonal) and of genetic (above diagonal) and phenotypic correlations (below diagonal) in the WL line.

| | afe | avgC | maxC | numC | gEgg | def |
|---|---|---|---|---|---|---|
| afe | **0.55 ± 0.02** | –0.06 ± 0.04 | –0.07 ± 0.04 | 0.01 ± 0.03 | –0.11 ± 0.04 | –0.03 ± 0.04 |
| avgC | –0.03 ± 0.01 | **0.34 ± 0.02** | 0.89 ± 0.01 | –0.92 ± 0.01 | 0.61 ± 0.03 | –0.20 ± 0.05 |
| maxC | –0.04 ± 0.01 | 0.62 ± 0.00 | **0.29 ± 0.02** | –0.87 ± 0.01 | 0.45 ± 0.03 | –0.04 ± 0.05 |
| numC | –0.02 ± 0.01 | –0.72 ± 0.00 | –0.6 ± 0.01 | **0.41 ± 0.02** | –0.28 ± 0.01 | 0.01 ± 0.05 |
| gEgg | –0.12 ± 0.01 | 0.45 ± 0.01 | 0.06 ± 0.00 | –0.42 ± 0.03 | **0.34 ± 0.02** | –0.78 ± 0.02 |
| def | 0.01 ± 0.01 | –0.23 ± 0.01 | –0.11 ± 0.01 | 0.11 ± 0.01 | –0.59 ± 0.01 | **0.20 ± 0.01** |
| $h^2_m$ | 0.41 | 0.37 | 0.21 | 0.39 | 0.14 | 0.09 |

Trait names as in Table 1, $h^2_m$—proportion of variance explained by markers.

selected for yolk to albumen ratio. From a sire and dam model in a commercial sire line, Akbas et al. (2002) estimated a heritability of 0.37 for clutch number and 0.22 for clutch length. For Box-Cox transformed clutch length, Chen and Tixier-Boichard (2003b) estimated heritability of clutch size and number around 0.4 in dwarf brown-egg layers. In a commercial layer line (LSL), the heritability of number of clutches and average clutch length were of 0.15 and 0.25 during 355 d of continuous observation using a transponder funnel nest (Icken et al., 2008).

The estimate of heritability of percentage of defective eggs in the RIR and WL lines was low (0.20) to moderate (0.28; Tables 2 and 3), which is similar to the estimate of 0.29 by Wolc et al. (2012) using a threshold animal model in a brown egg layer experimental line.

Egg production was genetically positively correlated with average (0.57 and 0.61) and maximum clutch size (0.61 and 0.45) and negatively correlated with clutch number (–0.55 and –0.28) and age at first egg (–0.24 and –0.11) in the RIR and WL lines (Tables 2 and 3). This confirms results of Chen and Tixier-Boichard (2003a) and Akbas et al. (2002), who observed an increase in egg number as correlated response to selection for increased average clutch length. Based on genetic parameters estimated in this study, despite higher heritability of clutch traits direct selection for egg number would be more efficient than increasing egg number through selection of any of the clutch traits with efficiency varying between 45% and 78%. The estimates of genetic correlations between egg number and clutch traits obtained by Bednarczyk et al. (2000) for Rhode Island White hens had a similar pattern in generation 1995/96 as obtained in this study but a positive genetic correlation (0.38) between egg number and clutch number in generation 1996/97. In a study with a transponder nest in a commercial layer line, estimates of the genetic correlation of clutch length with number of eggs and interval between clutches were of 0.64 and –0.57, respectively, during a 355-d recording period. Similarly, their estimates of the genetic correlation of number of clutches were negative with number of eggs (–0.53) and positive

**Table 4.** Chromosome (Chr) and position (Mb) of regions associated with egg production in the Rhode Island Red (RIR) and White Leghorn (WL) lines and their co-localization with known egg production QTL and genes involved in regulation of reproduction and light perception.

| Trait | Chr | Mb | Line | #SNPs | %Var | $P > 0$ | $P >$ average | QTL | Genes |
|---|---|---|---|---|---|---|---|---|---|
| afe | 1 | 60 | WL | 35 | 2.09 | 0.94 | 0.84 | AFE,EN, EPR,OWT | *WASH1* |
| afe | 2 | 90 | RIR | 67 | 1.16 | 0.93 | 0.77 | AFE | |
| afe | 4 | 84 | WL | 62 | 1.75 | 0.97 | 0.88 | | |
| afe | 8 | 2 | WL | 26 | 1.35 | 0.93 | 0.78 | | |
| afe | 11 | 5 | WL | 67 | 1.37 | 0.97 | 0.83 | EN | *SALL1* |
| afe | 26 | 4 | WL | 96 | 1.73 | 1 | 0.97 | | *PPARD* |
| afe | Z | 81 | RIR | 48 | 1.56 | 0.99 | 0.94 | | |
| avgC | 1 | 175 | RIR | 84 | 1.03 | 0.92 | 0.68 | | *RNF6, ATP8A2* |
| avgC | 6 | 28 | WL | 77 | 3.95 | 0.99 | 0.88 | | |
| avgC | 8 | 18 | RIR | 80 | 1.02 | 0.92 | 0.7 | EN | |
| avgC | 18 | 2 | WL | 132 | 1.6 | 1 | 0.94 | | |
| maxC | 2 | 4 | WL | 104 | 1 | 0.98 | 0.76 | | |
| maxC | 6 | 29 | WL | 33 | 1.79 | 0.9 | 0.73 | | |
| numC | 1 | 168 | RIR | 69 | 7.07 | 1 | 0.99 | | |
| numC | 2 | 31 | RIR | 39 | 2.36 | 0.95 | 0.91 | | *NPVF* |
| numC | 3 | 33 | WL | 45 | 1.18 | 0.95 | 0.85 | | *SRD5A2* |
| numC | 3 | 66 | WL | 45 | 1.55 | 0.97 | 0.87 | | *FOXO3, NR2E1* |
| numC | 6 | 28 | WL | 77 | 1.9 | 0.97 | 0.85 | | |
| gEgg | 3 | 10 | RIR | 121 | 2.2 | 0.95 | 0.75 | | |

Trait names as in Table 1, #SNP = number of SNPs within 1Mb window, %Var = percentage of genetic variance explained by the 1 Mb window, $P > 0$ = proportion of iterations of the Monte Carlo Markov Chain in which at least one SNP from that window had a non-zero effect, $P >$ Average = proportion of iterations in which the window explained more than the average proportion of genetic variance.

with clutch length (0.54), in the same recording period (Icken et al., 2008). Percentage of defective eggs did not show strong genetic correlations with clutch traits but it was negatively correlated with egg production (–0.58 and –0.75), as expected since only non-defective eggs were included in recorded production.

Phenotypic correlations between traits agreed in sign with genetic correlations but tended to be weaker (Tables 2 and 3). The phenotypic correlation between good egg production and maximum clutch length herein (0.11 and 0.06) were lower than the phenotypic correlations reported by Pavlidis et al. (2002) for two WL lines selected for low and high body weight of between 0.56 and 0.71. However, only clutches for up to the first month of production were measured in Pavlidis et al. (2002).

## Genome Wide Association Analysis

Proportion of variance explained by markers (marker based heritability) was lower than pedigree-based heritability estimates for all traits, except average clutch size in WL line (Tables 2 and 3). The extent of missing heritability was larger in the RIR than in the WL line. The difference between the lines was not explained by the small proportion of markers fitted in the model, which was tested by running models that included all markers (BayesA).

Genomic regions identified as associated with the analyzed egg production traits are in Table 4. Seven 1 Mb regions were associated with egg production traits in the RIR line and 12 regions in the WL line but there was no overlap between the lines. A region on chromosome 6 from 28 to 29 Mb was associated with number of clutches and with maximum and average clutch size in the WL line, suggesting a pleiotropic effect on temporal organization of the laying pattern. All other identified regions were trait specific. The largest number of associated regions was identified for age at first egg and no region exceeded the selected thresholds for percentage of egg defects. Age at first egg is also a trait that is well represented in the QTL database.

From the identified regions, the 60 Mb region on chromosome 1 overlapped with a region identified for age at first egg by Podisi et al. (2011) and Goraga et al. (2012), and was 3 Mb away from the QTL region reported by Tuiskula-Haavisto et al. (2004) for age at first egg. QTL for egg number (Hansen et al., 2005; Yuan et al., 2015), egg production rate (Sasaki et al., 2004), and ovarian weight (Sun et al., 2015) were also identified close to that location. The region on chromosome 2 (90Mb) overlapped a region identified by Podisi et al. (2011) for age at first egg, and the region on chromosome 11 was close to a QTL for egg number identified by Tuiskula-Haavisto et al. (2004). Of the regions associated with average clutch length, one in chromosome 8 (18 Mb) was close to a QTL for egg number reported by Tuiskula-Haavisto et al. (2004).

The 1 Mb regions identified in this study contained a total of 260 known genes. According to overrepresentation analysis in Panther, the only biological process that was significantly overrepresented after Bonferroni correction was the chemokine-mediated signaling pathway, with 12.6 fold enrichment and a *P*-value of 0.012. The connection of this immune-related pathway to egg production related traits is not clear. However, nine of the genes in the identified regions had gene ontology terms that were directly related to reproductive function or response to light stimuli:

*WASH1* (*ENSGALG00000012974*), which is involved in oocyte maturation; *RNF6* (*ENSGALG00000017105*), which is active in regulation of androgen receptor signaling and androgen receptor binding; *SALL1* (*ENSGALG00000003739*), which is involved in gonad development; *NPVF* (*ENSGALG00000011022*), which is involved in regulation of follicle-stimulating hormone secretion; *PPARD* (*ENSGALG00000002588*), which affects steroid hormone receptor activity and signaling; *SRD5A2* (*ENSGALG00000010625*), which is involved in the androgen metabolic process and male gonad development; *FOXO3* (*ENSGALG00000015297*), which is involved in oocyte maturation, ovulation from ovarian follicle, and antral ovarian follicle growth; *NR2E1* (*ENSGALG00000015305*), which is involved in steroid hormone receptor activity and visual perception; and *ATP8A2* (*ENSGALG00000017106*), which has a function in detection of light stimulus. Based on the key role of light stimulation for the onset and maintenance of egg production, these genes could be considered positional candidates for egg production and temporal organization of laying patterns. Confirmation of the role of these genes in regulation of egg production pattern requires further study.

## CONCLUSIONS

Estimates of heritability for multiple clutch traits in the RIR and WL lines were moderate. Positive genetic correlations were estimated between average and maximum clutch size and total saleable egg production. Genome-wide association analysis identified seven 1 Mb regions that were associated with egg production in the RIR line and 12 regions in the WL line. Except a region on chromosome 6 from 28 to 29 Mb, which was shared between number of clutches and maximum and average clutch size in the WL line, the regions were line and trait specific. Regions associated with egg production positionally overlapped with 260 genes, some of which were strong positional candidates for egg production based on gene ontology, including *WASH1*, which is involved in oocyte maturation, *NPVF* involved in regulation of follicle-stimulating hormone secretion, and *FOXO3* involved in oocyte maturation, and ovulation from ovarian follicle.

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