Genetics of rearing success in four pure laying hen lines during the first 17 weeks of age

I. Bouba,[*,†,1] H. van den Brand ◉,[‡] B. Kemp ◉,[‡] T. Bas Rodenburg ◉,[†,‡] and B. Visser ◉[*]

[*]Hendrix Genetics Research, Technology & Services B.V., 5831 CK Boxmeer, The Netherlands; [†]Animals in Science and Society, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, 3584 CL Utrecht, The Netherlands; and [‡]Department of Animal Sciences, Adaptation Physiology Group, Wageningen University & Research, 6700 AH Wageningen, The Netherlands

ABSTRACT This study aimed to investigate the genetics of rearing success (**RS**) in laying hens. Four rearing traits: clutch size (**CS**), first week mortality (**FWM**), rearing abnormalities (**RA**), and natural death (**ND**), were included as factors determining RS. Pedigree, genotypic, and phenotypic records of 4 purebred genetic lines of White Leghorn layers were available for 23,000 rearing batches obtained between 2010 and 2020. FWM and ND showed little or no variation amongst the 4 genetic lines over the years 2010−2020, whereas an increase was observed for CS and a decrease for RA. To determine whether these traits were heritable, genetic parameters for each trait were estimated, using a Linear Mixed Model. Heritabilities within lines were low (0.05−0.19 for CS, 0.01−0.04 for FWM, 0.02−0.06 for RA, 0.02 −0.04 for ND, and 0.01−0.07 for RS). Additionally, genome wide association study was done to scan the genomes of the breeders to reveal single nucleotide polymorphisms (**SNPs**) associated with these traits. Manhattan plots indicated the existence of 12 different SNPs having a significant effect on RS. Thus, the identified SNPs will increase the understanding of the genetics of RS in laying hens.

Key words: layer breeder, first week mortality, rearing success, heritability, SNP

INTRODUCTION

In laying hens breeding, after purebred line laying hen chicks have hatched, they stay at the rearing farms until approximately 17 wk of age, after which they are moved to the laying farms. Throughout these 17 wk, rearing remarks or abnormalities are registered and affected pullets are removed from the flocks. Besides these abnormalities, some pullets die a natural death (**ND**) during rearing. RS is defined as the percentage of animals that survived to the laying barn relative to the number of chicks that hatched from a batch. To investigate the genetics of rearing success (**RS**), clutch size (**CS**), first week mortality (**FWM**), rearing abnormalities (**RA**), and ND were used as determining traits. RS may be related with CS where a check originates from, that is, the number of eggs the female produces in each batch. CS has been shown to influence survival in wild birds

(Song et al., 2020). Nest survival of a wild swift's species during incubation was directly proportional to CS and there seemed to be an advantage to having larger CS during incubation (Pichorim, 2011). There could be a potential mechanism for CS in broilers or layers, but this has hardly been investigated. Of course, in commercial poultry, CS can be considered quite artificial, as breeder hens are laying eggs continually and CS only reflects the number of eggs laid in a specific period. Consequently, in this study, CS was defined as the number of eggs laid per laying hen in a period of 14 d. FWM is an important performance index as well as welfare indicator (Yerpes et al., 2020). FWM has been demonstrated to have a significant correlation with total mortality at the end of rearing for breeder flocks that showed a FWM exceeding 1% (Olsen et al., 2012). Furthermore, season has been shown to influence FWM in broilers ($P < 0.001$), where the highest mortality (on average 1.18%) was found from mid-March until mid-April, and the lowest mortality (on average 1.08%) from mid-September to mid-October (Koknaroglu and Atilgan, 2007; Yassin et al., 2009).

Another aspect that is hardly investigated in pullets is the genetics of RA as one of the contributing factors to RS. For example, Yeboah et al. (2019) focused mostly

on quality of day-old chicks with little attention for RA during the rest of their life. ND during rearing is another factor that has a strong negative correlation with RS and therefore of interest to study. It has been shown that ND in Lohmann Brown layers was 31.6% greater than culls due to inflammatory conditions, trauma or other reasons (Samkange et al., 2020). Furthermore, in a tropical savannah environment layer breeders ≥78 wk of age showed a lower risk of producing ND chicks compared to the younger chickens, with highest risk of ND for breeders between 19 and 38 wk of age (Shittu et al., 2014). Thus, it is anticipated that pullet flock age (**FA**) during rearing could influence RS. Both RS and its underlying factors are expected to be partly affected by genetics. Differences in genetic background is another factor that has been suggested to have an effect on survival in White leghorn pullets (Kaiser and Lamont., 2001). Brinker et al. (2018) investigated heritability of survival in 3 different layer hybrids and identified SNPs associated with survival time. The aim of this study was to investigate traits related to RS in layer breeder pullets to determine whether or not these phenotypic traits are heritable and additionally, to identify SNPs associated to RS. This will in turn allow for the selection of robust animals to improve welfare and production in the laying hen industry.

MATERIALS AND METHODS

Dataset

Phenotypes Hendrix Genetics, the Netherlands, provided a dataset of approximately 23,000 rearing batches from 4 purebred genetic lines of White Leghorn hens. Each batch contained eggs of 1 individual dam. Between 2010 and 2020, eggs per individual dam were collected over a period of 14 d and incubated thereafter. The dataset contained different categories of potential explaining factors: animal related factors, environmental factors, genetic factors and rearing factors. Animal related factors included 4 purebred genetic lines (**W1, W2, W3,** and **W4**) of laying hens, CS (the number of eggs laid by a dam within the period of 14 d), and embryo mortality (**EM**); the percentage of dead embryos during incubation relative to the number of eggs that was set from a batch at the start of incubation). Environmental factors included season; the period a rearing abnormality was registered and was categorized into 4 classes (December, January, February = Winter; March, April, May = Spring; June, July, August = Summer; September, October, November = Autumn), hatch week (the week in which the chicks were hatched with respect to year, for example, 20101 implies week 1 of 2010). Genetic factors included pedigree and genotypes. A rearing factor, pullet FA, age at which a rearing remark was recorded for a pullet in that flock. Rearing traits FWM, RA, ND, and RS whose heritabilities and SNPs effect were calculated.

RS is expressed as a percentage and calculated as

$$RS = 100 - (FWM + RA + ND) \qquad (1),$$

where FWM = first week mortality (percentage of pullets that died naturally in the first week), RA = Rearing abnormalities, which is the percentage of pullets that were removed from the rearing barn due to different remarks, ND = Natural death (the percentage of pullets that died naturally after the first week until 17 wk).

Genotypes For genome wide association study (**GWAS**) analysis, available SNPs and phenotypes were used. Hendrix Genetics provided genotypes of 1,748 dams; the maternal parents of the pullets described in this study. The dams were genotyped with the Illumina 60K SNP-chip for chickens. The exact number of genotyped individuals available for each genetic line was 460 for W1, 529 for W2, 411 for W3, and 348 for W4. After preprocessing, each genotype contained exactly 62,575 SNPs. No genotypes were available on the pullets themselves. Quality control on the SNPs was done in python 3.7.2, using pandas (McKinney, 2010; Reback et al., 2021). Furthermore, during the BLUPF90 analysis, a minor allele frequency threshold of 0.05% was applied for each genetic line (Marees et al., 2018). SNPs and individuals with a call rate less than 95% were removed. The Hardy-Weinberg equilibrium test for significance of 0.15 was used as exclusion criteria for the SNPs. Furthermore, the effect of sex chromosomes was taken into consideration by explicitly indicating them in the analyses. Variances were calculated for windows of 25 SNPs within a chromosome (Zhang et al., 2021). Manhattan plots were plotted in python 3.7.4, using bioinfokit, a bioinformatics data analysis and visualization toolkit (Bedre, 2021). All BLUPF90 analysis were performed in a high-performance computing terminal where the bash files were executed.

Methods

Across Population Analysis An unsupervised machine learning algorithm (K-means clustering) and principal component analysis (**PCA**) were integrated to examine the population structure of the genotypic data (Jansson et al., 2022). This was to determine the genetic differences or similarities amongst the 4 genetic lines. K-means clustering elbow method was used to learn the structure of the SNPs to determine the number of clusters that were suitable for the analysis (Humaira and Rasyidah, 2020). This step is important prior to determining whether or not factors affecting RS and the approach to calculate heritabilities should be done within or across genetic lines.

Factors Affecting RS Effects of FA, W, CS, EM, and season on RS were evaluated. Firstly, Pearson's correlations between these factors were calculated to ensure that they were not highly correlated to avoid the risk of including confounded factors. A P-value ($P \leq 0.05$) was used as a threshold for significant main effects. To investigate factors that affect RS, data was analyzed, using ordinary least square regression as shown in model (2):

$$y = \mu + FA + W + CS + EM + \text{ season} + e, \qquad (2)$$

Where y = rearing success, μ = population mean, FA = pullet flock age, W = genetic line (W1−W4), CS = clutch size, EM = embryo mortality, season = season (spring, summer, autumn, winter) and e = residual error. Conover's post hoc pairwise test for multiple comparison between genetic lines, using mean rank sum was applied (Pereira et al., 2015). Results are expressed as estimated means ± SEM. Bonferroni correction method was used as the threshold for the adjusted P-value during the post hoc analysis. All statistical analysis were performed in Python version 3.7.4 using statsmodels, scikit_posthocs and scipy libraries.

Heritabilities Prior to calculating heritabilities (h^2), a log transformation was performed on the traits of interest (CS, FWM, RA, ND, and RS) from the phenotypic data to bring them closer to a normal distribution. This step prepares the data for the model to be used in calculating h^2 (Mayhew and Meyre, 2017). In the phenotypic data, a zero observation did not mean a missing value, a FWM value of zero indicates that no mortality was recorded for that clutch in the first week. However, log (0) will give infinite values. To handle this, a scale factor of 1 was unanimously added to each of the values of these traits before transformation, because log (1) = 0, in this way the zero observations were preserved after the transformation (John and Paul, 2018). For each genetic line, h^2 for CS, FWM, RA, ND, and RS were calculated by fitting a single-trait linear mixed model (**LMMs**). Best Linear Unbiased Predictor (**BLUPF90**) family programs (Aguilar et al., 2019) were used, including RENUMF90 and Average Information Residual Maximum Likelihood (**AIREMLF90**). The RENUMF90 program provides data statistics, performs comprehensive pedigree checking (renumber the animal IDs from old to young in the pedigree file) and supports unknown parent groups. AIREMLF90 estimates variance components and after several iterations or rounds, the h^2 are calculated from variance components. In the first round, AIREMLF90 uses variance components specified in the parameter file as initial values (inferred from the statistical analysis in the previous section) to estimate variance components for the next rounds until convergence, a default convergence criterion of 1E-12 was used. A single-trait LMM model was used as shown in Equation (3):

$$y = X\beta + a + e \qquad (3)$$

where y is a vector of phenotypes, X is an incidence matrix of fixed effects (hatch week), β is a parameter vector of the fixed effects, a is the vector of breeding values and e is a vector of residuals. It is assumed that a and e vectors follow a normal distribution, $a \sim N$ $(0, \sigma_A^2 G)$ and $e \sim N$ $(0, \sigma_E^2 I)$, σ_A^2 is the additive genetic variance of the trait, σ_E^2 is the environmental variance of the trait, G is the genomic relationship matrix, I is the identity matrix. G is computed using the matrix of standardized genotypes Z as $G = ZZ'$

In addition, a bivariate LMM was run to consider traits that may be genetically correlated in case there is a gene influencing more than 1 trait.

GWAS To perform the GWAS analysis to obtain SNPs that have a significant effect on CS, FWM, RA, ND, and RS, only the genetic lines that showed highest heritabilities were considered given that they explain most of the genetic variance. Two additional BLUPF90 family programs, BLUPF90 (to generate SNPs P-values) and POSTGSF90 (to produce the solution file for the Manhattan plots) were used. Estimates of SNPs effect were obtained by back-solving (Strandén and Garrick, 2009) from the solutions to the vector of breeding values a in Equation 3. Bonferroni correction was used as a rejection threshold over multiple testing for significant SNPs effect (Aguilar et al., 2019), which equals 6.1 in our case on the − log10 scale. Thus, $P \leq 8\text{E-07}$ was used as a threshold for significant SNPs effect.

In the models of h^2 and GWAS hatch week was used as a fixed effect, however other factors like season and barn could influence the phenotypes under investigation. Hatch week was considered over season because it's a shorter period (the 2 are confounded).No observations were recorded on barn effect.

RESULTS AND DISCUSSION

SNPs Clusters

Figure 1A indicates that 4 clusters should be used to virtualize the population structure of laying hens as deduced from their SNPs data. Figure 1B shows a K-means clustering plot with PCA of the first 2 principal components of the SNP data, which together explained 69.70% of the SNPs variance. Because the 4 genetic lines have been kept reproductively isolated for many generations, a high degree of stratification (complete separation in 4 tight clusters of points) can be seen in the K-means PCA plot. From this, it can be inferred that the 4 genetic lines do not have recent common ancestors. This explains why in the next sections, statistical analysis was focused on making comparisons based on genetic lines and calculating heritabilities separately for each genetic line.

Statistical Significance

Four main effects, including CS, W, EM, and season showed a significant effect on RS (all $P \leq 0.001$). Figure 2A shows the relationship between EM and RS for 4 genetic lines. Clutches with EM <20% showed the highest RS irrespective of the genetic line, but above this value RS decreased and the decline varied among genetic lines. This deviation could also be due to the fewer number of observations in that region. Figure 2B shows the relationship between CS and RS for the 4 genetic lines. It was observed that RS increased with increasing CS. Larger clutches also showed less variation in RS between the genetic lines, implying that RS could be made more stable with larger CS, irrespective of the genetic differences that might exist between the genetic lines in the White Leghorn layer chickens. The deviation in RS between the genetic lines for smaller clutches (<8)
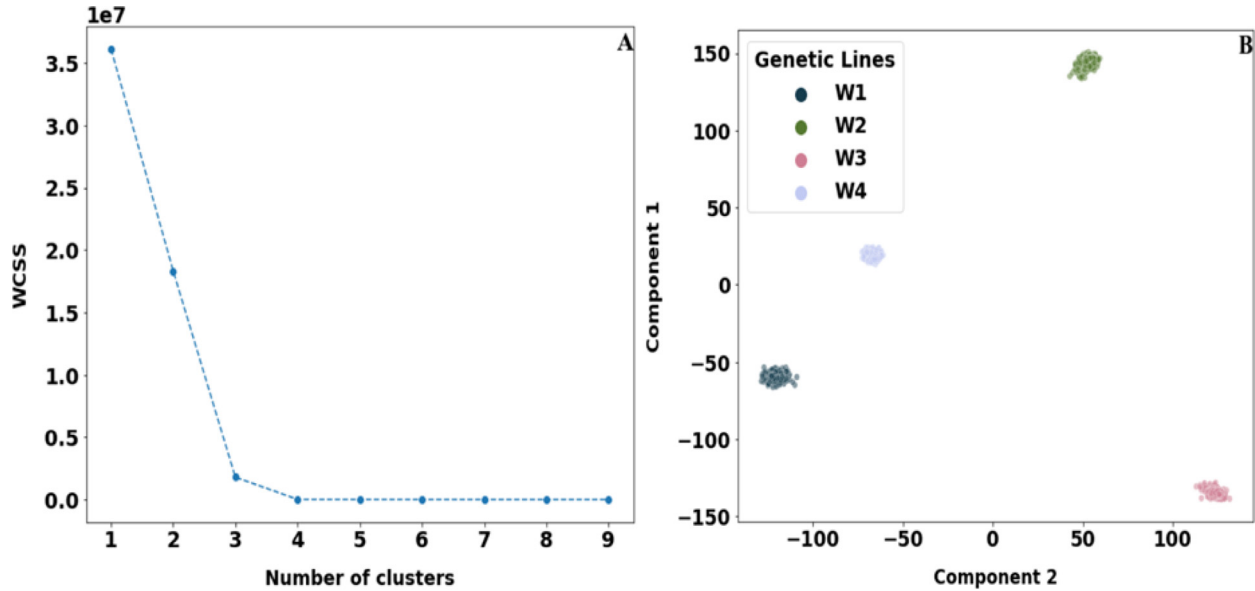
Figure 1. (A) K-means clustering elbow method of the SNPs data, **WCSS** is the sum of squared distance between each point and the centroid in a cluster, (B) K-means clustering plot of the first 2 principal components of the SNPs data.

might be due to the fewer number of observations. Despite season showing a significant effect on RS, there was little visible variation in RS between genetic lines with respect to season (see supplementary materials). This could be due to the large sample size of the data or because pullets were housed in temperature-controlled barns. Figure 3A shows the development in CS for 4 genetic lines over the years 2010−2020. All genetic lines showed an increase in CS, which might indicate progress in breeding selection for increased egg production. Figure 3B shows the development in RA for 4 genetic lines over the years 2010−2020. All genetic lines showed a decrease in RA over the years 2010−2016, but the decline was more pronounced in some genetic lines than in others (line W1 showed the strongest decline). The development of RA may differ between genetic lines across years because the pure-bred lines are genetically isolated, meaning that they have different allele frequencies, resulting in different phenotypic prevalence. Since

these populations are under constant ongoing selection, variation in RA over the years is expected. Between the years 2012 and 2016, fewer RA were recorded below 10 %, and this could be due a change in decision making over the criteria for taking birds out of the farm. For the remaining 2 traits (FWM and ND), limited difference over the years was observed.

Table 1 shows the average percentages for FWM, RA, ND, and RS per genetic line (W1, W2, W3, and W4). While the genetic lines did not show any significant difference on FWM, they showed an effect ($P < 0.001$) on RA, all lines affected RA differently with W3 recording the highest percentage of RA followed by W4, W2 and W1. ND was less affected ($P < 0.03$) by all genetic lines, unexpectedly W2 did not differ from W1, but it differed from W4, this might be related to relatively fewer number of observations (only 4,211 for W4). Genetic lines showed a significant effect ($P < 0.001$) on RS, but the effect varied amongst lines, W2 and W3 showed a
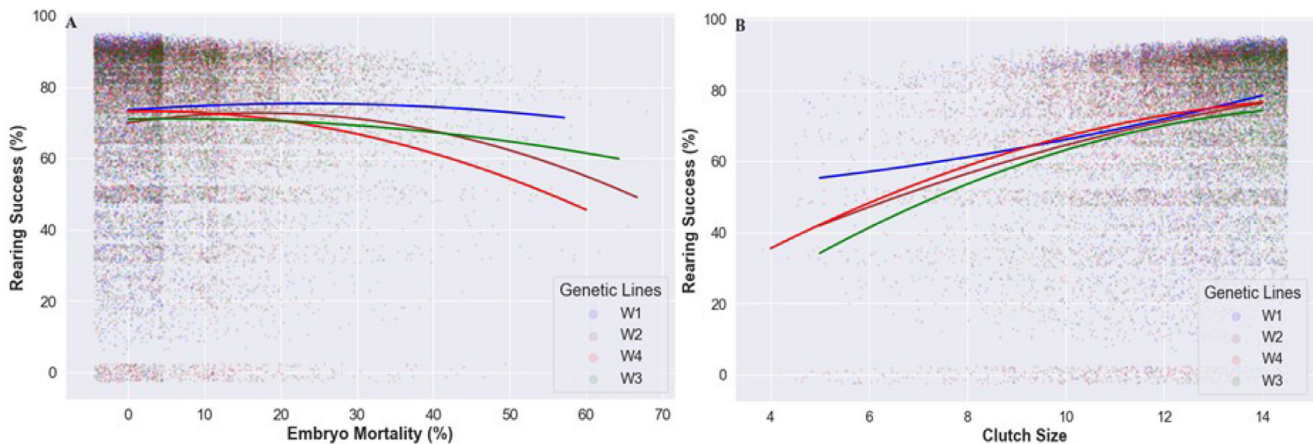

Figure 2. (A) Relationship between embryo mortality during incubation and rearing success, (B) Relationship between clutch size during a 14-d egg collection period and rearing success. Each dot represents a batch of eggs. The length of the horizontal regression fits falls within the boundary of the original data points. Dots appearing beyond this boundary were because of the jitter effect that was applied to disperse overlapping data points. This was to improve visualization and did not have any effect on the results.
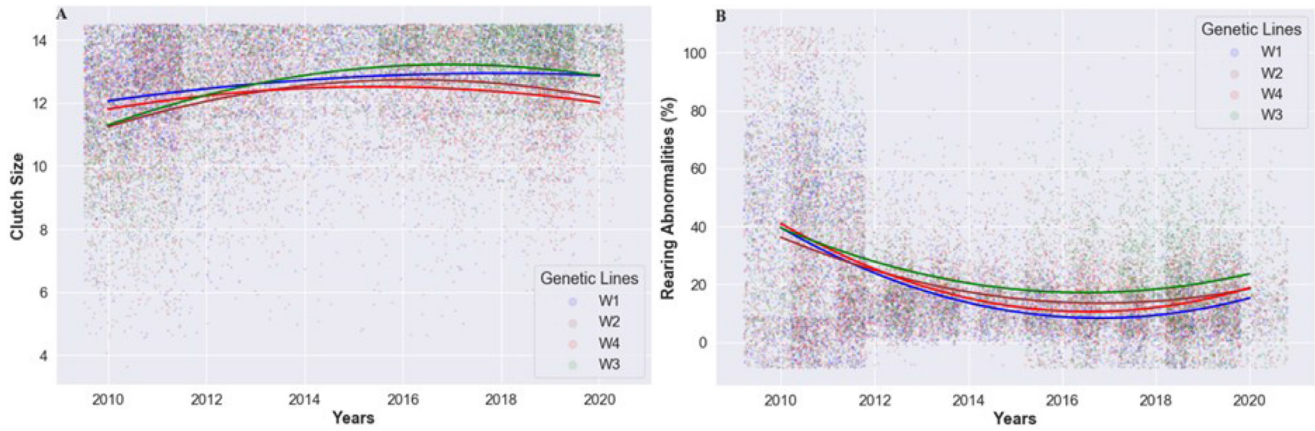
Figure 3. (A) Trend of clutch size over the years (2010−2020) for 4 purebred genetic lines of laying hens, eggs were collected over a period of 14 d, thus maximum clutch size is 14, although in the figure it appears to be more, it was because of the jitter effect that was applied to disperse overlapping data points. This was done to improve visualization and did not have any effect on the results. (B) Development of rearing abnormalities over the years (2010−2020) for 4 purebred genetic lines of laying hen chickens. Data points on the y-axis fall between (0−100)% although in the figure they appear beyond, it was also because of the jitter effect that was applied. In both figures (A and B) each dot represents a batch of eggs, the length of the horizontal regression fits falls within the years (2010−2020).

significant difference in their effect on RS, whereas W1 and W4 showed no difference. Finally, pullets from genetic line W1 showed the highest RS (Δ 2.44%) and these results are complementary to those visualized in Figures 2A and 2B.

Calculated Heritabilities (h^2)

Pearson's correlations between CS, FWM, RA, ND, and RS were as follows: FWM: -0.27, RA: -0.85, ND: -0.17, CS: 0.24. Thus, these 4 traits were explored further as potential explainers of RS. Table 2 shows the h^2 of CS, FWM, RA, ND, and RS for each genetic line from the single-trait LMM. The h^2 from the bivariate LMM were close to zero, thus the results were not included. For CS, genetic line W4 showed the highest h^2 (0.19) and this value falls within the range of h^2 reported on similar studies in different species of chickens. Moderate h^2 (0.29−0.41) were estimated for CS in White Leghorn commercial lines (Wolc et al., 2019), whereas h^2 was 0.11 to 0.23 in Rhode Island white commercial lines (Wolc et al., 2010). Furthermore, in wild birds' population, a h^2 of 0.24 for CS was reported in great tits (*Parus major*) (Evans et al., 2020). In wild birds, parents provide food to the young, thus an optimal CS will result in the maximum number of surviving chicks (Liu et al.,

2018), whereas in laying hens, CS is quite artificial and not fixed (determined by the number of eggs laid in a particular period). Thus, the h^2 of CS in wild birds is more crucial to survival than in laying hens that are commercially produced with unlimited amount of feed.

For FWM, genetic line W1 indicated the highest h^2 (0.04). A study in purebred broiler chickens of 20 overlapping mating groups from multiple breeder flocks showed mortality rate peaks in the first week after hatch and gradually peaks again after week 7 (Zhang et al., 2018) and the same study reported a h^2 (0.13) for mortality in these broiler chickens. A decrease in early life chick welfare could be reflected in high first-week mortality and some breeds are prone to higher FWM due to genetic factors (Yerpes et al., 2020). For RA, genetic line W2 indicated the highest h^2 of 0.06. The most

Table 1. Average percentages of first week mortality (FWM), rearing abnormalities (RA), natural death during rearing (ND), and rearing success (RS) for 4 different genetic lines (W1−W4) over the years 2010−2020.

Genetic line	N[1]	FWM (%)	RA (%)	ND (%)	RS (%)
W1	5,570	1.64	21.14[d]	3.29[b]	73.92[a]
W2	6,759	2.81	21.64[c]	4.40[a]	71.14[c]
W3	7,016	1.73	24.43[a]	3.14[b]	70.70[c]
W4	4,211	2.08	21.90[b]	3.44[b]	72.59[b]
SEM		0.04	0.14	0.05	0.14
P-value		0.94	<0.001	<0.03	<0.001

[1]Number of batches.
[ab]Estimated means within a column lacking a common superscript differ (*P* < 0.05).

Table 2. Heritabilities for clutch size (CS), first week mortality (FWM), rearing abnormalities (RA), normal death (ND), and rearing success (RS) for 4 different genetic purebred lines (W1 −W4) over the years 2010−2020.

Trait	Genetic line	h^2 (SE)
CS	W1	0.05 (0.02)
	W2	0.08 (0.02)
	W3	0.05 (0.02)
	W4	0.19 (0.03)
FWM	W1	0.04 (0.01)
	W2	0.03 (0.01)
	W3	0.01 (0.01)
	W4	0.01 (0.01)
RA	W1	0.02 (0.01)
	W2	0.06 (0.02)
	W3	0.04 (0.02)
	W4	0.03 (0.02)
ND	W1	0.03 (0.01)
	W2	0.02 (0.01)
	W3	0.04 (0.01)
	W4	0.02 (0.02)
RS	W1	0.01 (0.01)
	W2	0.07 (0.02)
	W3	0.01 (0.01)
	W4	0.04 (0.02)

Standard errors in parentheses.

prevalent RA in this study were crooked toes (4.01%), splayed legs (2.03%), and poorly developed birds (1.82%) in total. For ND, genetic line W3 showed the highest h^2 (0.04) and in general, there is hardly any study that directly investigated the relationship between ND and RS. For RS, genetic line W2 showed the highest h^2 (0.07), recognizing heritable components can increase response to selection, which is in line with Alemu et al. (2016) who reported also a low h^2 (0.02 −0.10) for survival in brown laying hens.

SNPs Effect

Figures 4A−D show Manhattan plots of CS, FWM, ND, and RS, respectively for those genetic lines that showed the highest heritabilities for these traits (Table 2). There were no significant SNPs for RA, hence no Manhattan plot is shown for this trait. The y-axis of

the Manhattan plots represents minus the logarithm in base 10 of the P-value of the effect of each SNP (Deelen et al., 2019), whereas the x-axis indicates the base-pair position of each SNP within a chicken's chromosome. On each of the Manhattan plots, the extrapolated horizontal line marks the thresholds for SNPs with a significant effect.

For all traits, the percentage of variance explained by a region of 25 adjacent SNPs was in the range 0.001 to 0.08%. Eight SNPs on chromosomes 1, 4, 5, 12, and 28 were observed to have a significant effect on CS. GWAS studies on white leghorn hens have identified 12 regions that were line and trait specific to CS (Wolc et al., 2019). A similar study (Wolc et al., 2010) in a population of Rhode Island White hens, indicated an opportunity of selection aimed at improvement of egg production persistence, by an increase in the average CS. The higher number of significant SNPs might be due to CS being a reproductive trait (Shad et al., 2013)
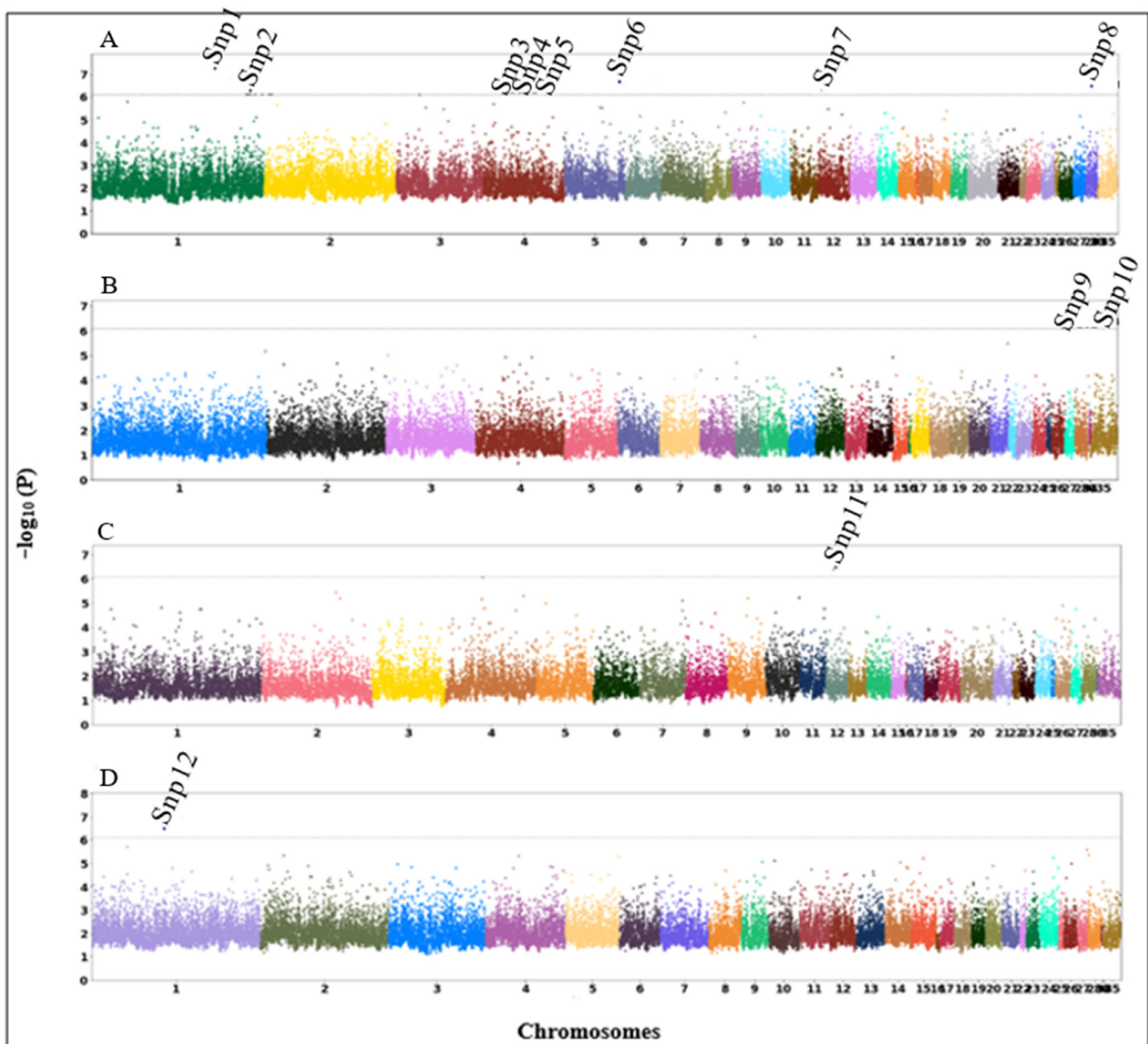

Figure 4. Manhattan plots of significant ($P \leq$ 8E-07) SNPs. (A) clutch size for genetic line W4, (B) first week mortality for genetic line W1, (C) natural death for genetic line W3, and (D) rearing success for genetic line W2. Each dot represents a SNP from the chicken's chromosome.

and could also be polygenic (Zhao et al., 2021). Two SNPs on chromosomes 26 and 35 were observed to have a significant effect on FWM. One SNP on the chromosomes 12 and 1 were observed to have significant effects on ND and RS, respectively. However, no significant SNP was identified for RA. Confirmation of the role of these SNPs in the regulation of RS will require cross referencing with annotation databases and will be explored in our next study.

It can be concluded that CS, FWM, RA, and ND are relevant traits in investigating RS. This study shows that heritabilities of FWM, RA, ND, and RS are low, except for CS which had a higher heritability. A batch of chicks that hatched from a larger CS showed a higher RS (the more eggs a dam lays within a period of 14 d, the better the survival of her chicks). The genotypic information revealed that the purebred lines have a well separated population structure. The significant SNPs in the Manhattan plots may indicate the existence of genes of a large effect. Possibly, major genes can be identified via fine mapping of the genome around the region of the SNPs. All traits except RA, showed SNPs with significant effects. The identified SNPs will increase the understanding of the genetics of RS in laying hens. This might lead to increased benefits in production and decreased stressful conditions during rearing, thus, improved welfare. As a recommendation, tissue samples of affected hens should be collected for genotyping to provide a better and traditional case-control GWAS investigation. These would give a greater statistical power to identify causal variants.

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DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2023.102576.

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