Effect of Endogenous Retroviral Elements (ALVE) on Egg Size in Commercial Egg production lines

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Introduction

The genome of the chicken contains endogenous viral (ev) elements that are closely related to the Avian Leukosis Virus (ALV) retrovirus. During the evolution of an avian genome, retroviruses infect cells and insert their DNA into the host genome. These ALV subgroup E (ALVE) elements are then stably inherited and passed on to subsequent generations. The ALVE elements can continue to retrotranspose within the genome and insert into or nearby genes and affect gene function. Multiple ALVE elements related to the avian leukosis virus have been identified in the chicken genome and some have been shown to have specific phenotype effects. The recessive white phenotype is due to a retroviral insertion (ALVE-TYR) in the tyrosinase gene (Chang et al 2006) and the sex-linked slow feathering phenotype used for determining sex of day old chicks is associated with ALVE21 insertion (Bacon et al 1988). Both positive and negative impacts of retroviral elements have been reported for multiple traits including body weight, egg production, egg weight and resistance to Marek's disease (Gavora et al 1991; Iraqi et al 1994; Kuhnlein et al 1989).

With the availability of relatively inexpensive sequencing techniques, genomes can now be analyzed and the presence of ALVE elements can be detected based on sequence homology with retroviruses. Utilizing sequence information on ALVE elements, novel PCR-based detection assays were developed for 20 ALVE inserts detected in 8 lines used for commercial production of both white shell and brown shell eggs (Mason, 2017). Multiple generations of males (500-1500 per line) were genotyped for the subset of 17 ALVE inserts that were segregating across the lines. Each male had daughter performance records. An association test between the presence of each ALVE insert and three performance traits affecting egg weight was performed.

Materials and Methods

Genetic Material and Traits

DNA was tested from multiple generations of 8 lines of males from three different breeds; five White Leghorn (WL), two White Plymouth Rock (WPR) and one Rhode Island Red (RIR). The average of the daughter phenotypes for each sire was used as the phenotype value. Not all traits were measured for all generations. The number of genotyped males ranged from 500-1500 per line. Traits examined in this study included weight of the first 3 eggs (E3, g), average egg (EW, g) and yolk weight between 25 and 45 weeks of age (YW, g).

Genotyping

PCR assays for 20 ALVE inserts were developed for the region surrounding the insert from genomic sequence information, and sequence of each specific insert. Some of these ALVE inserts had been previously reported. Novel detection primers were developed for all ALVE inserts using competitive allele specific PCR chemistry (KASP; LGC, Middlesex, UK). The primers were designed to detect the presence or the absence of each ALVE insert thus allowing for the positive identification of both homozygotes and the heterozygote. Each ALVE assay was genotyped on DNA from only those lines in which they were segregating. Genotype data was generated for 17 ALVE inserts and analyzed using Kraken software (LGC, Middlesex, UK).

Association Tests

All genotype classes with less than 5 observations were removed prior to the analysis. The association analysis was performed using lm procedure in R (R Core Team, 2015). In the first step, a model with fixed effects of generation, ALVE genotype and their interaction was fitted for each trait and line separately. For traits with a significant ($p<0.05$) main effect of SNP and a non-significant ($p>0.1$) SNP by generation interaction, the interaction was dropped from the model and number of ALVE gene copies was fitted as a regression in order to estimate additive ALVE gene effect.

Results and Discussion

Significant associations between ALVE genotype and the 3 egg weight related traits (E3, EW, YW) were found in 5 of the 8 lines and for 7 of the 17 ALVE. Table 1 summarizes the significant associations and the size and direction of the effects.

Table 1. Statistical significance of ALVE inserts on 3 egg weight traits and the size and direction of those effects.

		Statistical Significance				Size and Direction of Effect ± se, (grams)		
LINE	**ALVE insert**	**E3**	**EW**	**YW**		**E3(g)**	**EW(g)**	**YW(g)**
WL3	ALVE3	NS	*	NS			-0.11 ±.053	
WL6	ALVE3	*	NS	NS		0.18 ±.075		

RIR1	ALVEB5	***	NS	NS		-0.28 ±.080		
WPR1	ALVE-ros004	NS	NS	*				0.14 ±.061
RIR1	ALVE-ros004	NS	***	NS			-0.32 ±.073	
RIR1	ALVE-ros006	***	***	NS		0.41 ±.078	0.38 ±.081	
RIR1	ALVE-ros007	NS	NS	**				-0.06±.020
WPR2	ALVE-ros009	NS	***	**			-0.38 ±.069	-0.07±.022
WPR2	ALVE-NSAC7	NS	**	NS			-0.23 ±.088	

NS=non-significant; *=p<0.05; **=p<0.01; ***=p<0.001

Only those associations that were significant were included in the table, and the size with standard error (se) in g and direction of the effect on the trait per copy of the ALVE insert are also included. ALVE3 was segregating in 4 of the 8 lines tested but a significant (p<0.05) effect was found in only two of the lines. The presence of ALVE3 had a negative effect on EW in WL3 and a positive effect on E3 in WL6. ALVEB5 was segregating in the WPR and RIR lines, but showed a highly significant (p<0.001) and negative impact of 0.28g on E3. ALVE-ros004 had significant (p<0.05) and positive effect on YW in one of the three lines in which it was segregating (WPR1) and a highly significant (p<0.001) and negative effect on EW in RIR1. ALVE-ros006 segregated in RIR1 only and had a highly significant (p<0.001) and positive effect on both E3 and EW. ALVE-ros007 segregated only in RIR1 and had a significant (p<0.01) negative impact of 0.06g on YW. ALVE-ros009 was segregating in 2 of the lines, but an effect of the insert was seen only in WPR2, where it was highly significant (p<0.001) for EW resulting in a 0.38g lower egg weight, and a significant (p<0.01) lower YW (0.07g). The ALVE-NSAC7 was segregating in two of the lines, but had a significant effect on EW only, resulting in a 0.23g lower egg weight.

The impact of ALVE inserts reported here are consistent with previous reports in the literature which determined that ALVE inserts are associated with egg size (Gavora et al 1991). However, depending on the specific ALVE insert tested, the results presented here show that the effect could result in either increased or decreased egg size or have no effect. Previous studies detected the presence of ALVE inserts utilizing hybridization of genomic DNA with exogenous ALV genome probes (Kuhnlein *et al* 1989). The PCR based detection method used herein is more precise and can detect specific insertions of even partial ALVE sequences. More ALVE inserts can now be detected, and it is possible that these smaller and partial ALVE inserts can have less deleterious impact than the longer previously detected ALVE inserts. While the presence of ALVE genes has been shown in the past to have negative fitness impacts due to expression of viral antigens, these partial inserts may not produce any viral proteins. These ALVE inserts may not impact the traits directly, but may simply be genetic markers for nearby quantitative trait loci (QTL).

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