Identification of Quantitative Trait Loci (QTL) for Production Traits in Japanese Quail (*Coturnix japonica*)

In the current study, growth performance and nonlinear growth curve functions, quantitative trait loci (QTLs) for growth- and egg-related traits of large- and normal-sized (LS vs. NS) Japanese quail (*Coturnix japonica*) were investigated. Growth- and egg-related traits are the most important economic traits in poultry breeding industry. Bird growth is a vital quality criterion involving changes in the size, shape, weight, and volume of the body. Growth variation was evaluated by measuring body weight and fitting growth curve models to these measurements. Growth curve models furnish useful information on bird management and health, age at sexual maturity, appropriate slaughter age, and the effects of genetic improvements. Quail eggs are a suitable source of animal protein fulfilling the nutritional requirement of the growing world population, because it contains all the essential amino acids for humans and provides significant amount of several vitamins and minerals. External and internal egg-related traits are the most important egg traits that influence the quality of eggs, hatching performance, body weight of newly hatched chicks, propagation of the flocks, and finally breeding's economy of poultry industry. Production traits in poultry are complex traits controlled by multiple QTLs and influenced by genetic and environmental factors and their interactions. QTL analysis for genetic bases of variation can increase the response of breeding programs via investigation of genotype and phenotype relationship basis using genomic DNA markers. In QTL analysis, the chromosomal position of useful loci is identified based on DNA markers especially single nucleotide polymorphisms (SNPs) markers. Next-generation sequencing (NGS) technologies brought new advances in genotyping by sequencing (GBS) methods and related bioinformatics computing resources. This progress caused to facilitate the large-scale discovery of SNPs and made it in a rapid and cost-effective manner. Restriction-site associated DNA sequencing (RAD-seq) is one of the GBS methods that can identify, verify, and score thousands of SNPs simultaneously; reduce complexity across genome; deliver high resolution population genomic data; and is convenient for non-model species at a reasonable cost. The current investigation was designed to detect QTLs underlying growth- and egg-related traits in Japanese quail using the RAD-seq. In general, 805 birds were used to construct the resource family for QTL analysis. Large-sized (LS) and normal-sized (NS) quail strains were selected as parental generations. LS and NS quail were reciprocally crossed. The offspring obtained from the cross between a LS male with three NS females, and a NS male with three LS females were called A and B, respectively. One hundred quail from each of the LS and NS strains, 50 birds from each of their reciprocal F_1

generations (A and B), 277 (138 females and 139 males) F_2 from F_1 A and 228 (111 females and 117 males) F_2 from F_1 B were used in this study. Based on these, three studies were performed.

Body weight and nonlinear growth curve analysis

The current investigation was organized to compare the nonlinear regression growth models and to fit the best predictive function using the live body weight data of the reciprocal F_1 birds mentioned above. Body weight was measured weekly from hatching to 16 weeks of age. In this study, Weibull, Logistic, Gompertz, Richards and Brody growth models were used to determine the function that best defines growth. According to the goodness-of-fit criteria (higher Adj. R^2 and lower AIC and BIC), the Richards model showed the best fit in LS females and males (Adj. R^2 = 0.999, 0.999, and AIC = 92.007, 81.742, and BIC = 96.173, 85.908, respectively). On the other hand, the Gompertz exhibited the best fit in the NS quail (Adj. R^2 = 0.999, 0.993, and AIC = 67.341, 79.516, and BIC = 70.674, 82.849, in females and males, respectively). The F_1 A birds presented the best fit to the Richards. On the contrary, F_1 B quail expressed the best fit to the Gompertz function. Weibull and Logistic exhibited the good fit next to the Richards and Gompertz. While, Brody model presented the poorest fitability to the curve. As a result, both Gompertz and Richards were highly fitted models to describe growth in Japanese quail. Moreover, the growth pattern of the F_1 birds was suggested to be inherited from the male parental strain. To my best knowledge, this is the first study that compared growth curves in the reciprocal F_1 birds and their parental quail strains. These results will be effective for the QTL analysis of growth curve parameters.

Identification of QTL for growth-related traits

This investigation was designed to identify QTLs for growth-related traits by constructing a genetic linkage map based on SNP markers using RAD-seq in Japanese quail. A QTL mapping population of 505 F_2 birds was used as materials. Body weight was measured weekly from hatching to 16 weeks of age. Five nonlinear regression growth models were analyzed, and the growth curve parameters of Richards and Gompertz were selected as the best model for describing a quail growth curve of the F_2 in A and B families, respectively. RAD-seq developed 125 and 109 SNP markers that were informative between their parental strains in A and B families, respectively. Map Manager QTX b20 software constructed 16 linkage groups of the SNP markers that spanned 795.9 centiMorgan (cM) with an average marker interval of 7.3 cM in A family, and 21 linkage groups of the SNP markers that spanned 640.0 cM with an average marker interval of 7.3 cM in B family. QTL analyses of phenotypic traits using R/qtl package revealed 4 main-effect QTLs in A family and 7 main and 2 epistatic QTLs in B family. Detected QTLs were located on chromosomes 1 and 3 in A family and 2, 3, 5, 9, 19, and 20 in B family associated with all BW

traits at genome-wide significant at 1% and 5% and suggestive at 10% levels. No QTL was detected for BW in the second week of age. This is the first report that identified QTLs for asymptotic weight and weight at inflection point of the Gompertz parameters, and asymptotic weight, and age and weight at inflection point of the Richards parameters in Japanese quail. These results highlighted the combination of QTL studies and the RAD-seq method in Japanese quail and will aid future breeding programs for identifying genes underlying QTLs and in the application of marker-assisted selection.

QTL mapping on external and internal egg-related traits

This research was conducted to identify QTLs associated with egg-related traits by constructing a genetic linkage map based on SNP markers using RAD-seq in Japanese quail. A total of 2490 eggs were investigated from a QTL mapping population of 249 F_2 females at two different stages, at the first stage of egg-laying and 12 weeks of age. Five eggs were analyzed at each stage. RAD-seq developed 118 SNP markers in A family and 100 SNP markers in B family, and the markers were mapped in 13 and 17 linkage groups in each family, using Map Manager QTX b20 software. The markers spaned 776.1 cM in A family and 628.6 cM in B family with an average spacing of 7.4 and 7.6 cM in each family. Main-effect QTLs in A and B families (9 vs. 2 QTLs, respectively), for 27 phenotypic traits in each, were identified using "Simple interval mapping" in R/qtl package. Main-effect QTLs included one for each yellowness of yolk color at the age of 12 weeks and age at first egg on chromosome 2, one for egg long axis at the first stage of egg-laying on chromosome 4, one for each egg short axis and yolk weight at the first stage of egg-laying on chromosome 6, one for egg weight, egg short axis, albumen weight, and yolk weight at the first stage of egg production on chromosome 10, one for egg shell weight at the first stage of egg-laying on chromosome 12, and one for each egg short axis and yolk diameter at the first stage of egg-laying on Z chromosome in A family. In B family, one QTL for lightness of yolk color at the first stage of egg production on chromosome 7, and one for lightness of yolk color at 12 weeks of age on chromosome 12 were identified. QTLs were detected at genome-wide significant at 1% and 5% and suggestive at 10% levels. This is the first study that performed QTL analysis on egg-related traits using RAD-seq.

In general, the findings of this investigation indicated that growth- and egg-related traits are regulated by many QTLs at different growth stages associated with production in breeding programs for poultry industries. The findings are useful for developing the Japanese quail QTL database as well as applying to other Galliformes species, especially chickens. These results highlighted the effectiveness of RAD-seq associated with QTLs targeted and the application of marker-assisted selection in the poultry industry, particularly in Japanese quail.