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Original Article

Association Analysis Between ESR Gene and Egg Production Performance of Laying Quails

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■Keywords

Association analysis; egg quail; ESR gene; egg production performance; PCR-RFLP.

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ABSTRACT

In order to explore the effect of estrogen receptor (ESR) gene polymorphism on the egg production performance of quails, the polymorphisms of exon 1, exon 4 and exon 8 of the ESR gene in three Laying Quail Populations of Chinese Yellow quail, Korean quail and Beijing white quail were detected by PCR-RFLP, and their association with the egg production performance of quails was analyzed. The results showed that three genotypes were detected in exon 1, exon 4 and exon 8 of the ESR gene in three quail populations, namely TT, CC and CT genotypes. Among them, the frequency of CC genotype in exon 1 of the ESR gene was the highest in Chinese Yellow quail, Beijing white quail and Korean quail (0.515, 0.600 and 0.723); Exon 4 of the ESR gene had the highest frequency of TT genotype (0.409, 0.617) in Chinese yellow and Korean quails, while CC genotype was the highest in Beijing white quails (0.667).The frequency of TT genotype in exon 8 of the ESR gene was the highest in Beijing white quail and Korean quail (0.708, 0.500), while the frequency of CT gene was the highest in Chinese yellow quail (0.521). The results of association analysis showed that there was no correlation between exon 1 of the ESR gene and quail egg production performance (p>0.05). Exon 4 of the ESR gene was significantly correlated with laying traits such as feed-egg ratio, egg laying number, laying rate and starting weight of quails (p<0.05). Exon 8 of the ESR gene was significantly correlated with feed-egg ratio, egg laying number and egg laying rate of quails (p<0.05).In conclusion, the research shows that the ESR gene can be used as a candidate gene for marker assisted selection of egg production performance of Laying Quails, which provides a theoretical basis for cultivating new egg laying quail lines with better egg production performance.

INTRODUCTION

The estrogen receptor (ESR) gene was discovered by Jensen *et al*. (1962). It mainly affects the secondary sexual characteristics, fertility, reproductive cycle and pregnancy maintenance of female animals, as well as the development and systematic differentiation of embryos. The estrogen receptor gene is a member of the nuclear receptor superfamily, which is mainly located in the nucleus. ESR is a transcription factor. After specific binding with estrogen in animals, it obtains a complex. This complex is a hormone receptor complex, which acts on the receptor response element in the front of the target gene. It can induce the transcription of specific target genes and trigger a gene regulation mechanism, inducing estrogen to produce multiple effects. There are two subtypes of estrogen receptor, ESR α (esr-1) and ESR β (esr-2), which are encoded by different genes. As an important factor in the breeding process of livestock and poultry, ESR α Genes play a leading

role in breast and uterus, while ESR β mainly affects the central nervous system, cardiovascular system, immune system, urinary system, kidneys, bones and lungs (2004).

Intensive quail breeding is a key element of modern poultry breeding in China. At present, there are more than 1 billion quails in the world, and about 200 million quails in China (2005). It is the largest country production of quails in the world. After nearly 20 years of stable development, the quail industry in China has become the "second poultry industry", after the chicken industry (2002). Quail has the advantages of a small size, fast growth, less investments, less material consumption, and high profits. In recent years, the vigorous development of molecular marker genetics and breeding has added new vitality and broad development prospects to the quail industry. The international poultry industry recognizes that the quail industry will become its pillar industry in the 21st century.

There are many factors that can affect the egg production performance of quails, such as the environment, temperature, feeding conditions, etc., and the heritability is low. The speed of quail breeding through conventional breeding is relatively slow. At present, the research on molecular marker genetics and breeding is very popular, and it has become a new technology for variety breeding and improvement. Many scholars have carried out correlation analyses between many ESR genes and reproductive performance in pigs, chickens, cattle, sheep, guinea pigs and other animals Zhang *et al*. (2013). Lu *et al*. (2016) studied the polymorphism of the ESR gene in pigeons for the first time, and analyzed its correlation with egg production, concluding that in ESR β, the S8 locus was significantly correlated with egg production of Taiping king pigeons. Wang *et al*. (2012) have determined that the CC type of ESR gene has a significant impact on the laying performance of chickens, but whether it can be used as an important theoretical basis for breeding of Zhuanghe big bone chickens remains to be studied further. Chen *et al*. (2012) found that the five genotypes in the chicken ESRl gene Inl segment were highly polymorphic, and the population genetics deviated from the Hardy Weinberg equilibrium ($p<0.05$). The analysis of variance showed that the early egg laying traits among individuals of each genotype reached a significant ($p<0.05$) or extremely significant ($p<0.01$) level, and the individual performance of T1T3 genotype was superior. Artificial selection of this population could improve egg laying performance. Therefore, ESR gene polymorphism can be used as an auxiliary marker for quail breeding.

If that is the case, we could carry out marker assisted selection of the ESR gene as soon as quails are born, so as to achieve the purpose of early seed selection, being able to timely eliminate quails with low egg production performance and choose quails with good egg production performance. After being implemented in production, this process can reduce the rising cost of quail, create more economic benefits for quail breeding companies, and promote further development of the quail industry.

MATERIALS AND METHODS

Strains and Feeding Management of Quail

In this study, three egg laying quail varieties with different egg laying performances were selected as the research object. The average egg weight of Korean quails was the largest, while the total egg weight was medium; the average egg weight of Chinese yellow quails is the smallest, while the total egg weight is the largest; and the egg weight of Beijing white quails is slightly small, but the total egg weight is the smallest. These three varieties of experimental animals were raised for 1-17 weeks, with 50 quails of each type, 150 in total. After 2 weeks of age, the quails were raised in separate cages. Each cage contained 10 quails of the same variety, 2 males and 8 females. Quails of the same variety in the same cage mated freely, and the legs of each quail were labeled.

Blood collection and genomic DNA extraction

After 17 weeks of feeding, the quails were slaughtered, blood samples were collected, and the genomic DNA was extracted using a kit method. The whole blood genomic DNA Extraction Kit for poultry was provided by Bioteke Corporation. During the experiment, the quails were in good health.

Primer synthesis of ESR gene

The primer sequence of the ESR gene is shown in Table 1 Pu. (2016). Exon 1, exon 4 and exon 8 of the ESR gene were synthesized by Beijing DingGuo ChangSheng Biotechnology Co., Ltd.

Table 1 – Primer sequence information.

Name	Primer Sequence(5'-3')	Size(bp)	Annealing Temperature(°C)
ESR-E1	F: CAAAGCCTCTGGAGTTAC R: AGCAGTTTCCCTCATCCC	370bp	55.4
ESR-E4	F: CGGGCGAATGATGAAACA R: CCCAGTTGATCATGTGCA	301bp	58.0
ESR-E8	F: CAACAAAGGAATGGAGCA R: CCCTTCTTTTGCTGTTAA	212bp	53.6

Performance measurement

The traits and methods used in egg production performance measurement were as follows:

Egg laying rate (%): refers to the percentage of quails laying eggs on the statistical date

Egg laying rate = (total number of eggs laid in the statistical period) / (number of quails in the same cage) × Statistical date (number) × 100

Feed-egg ratio: food intake / total egg weight.

Feed-egg ratio = total feed weight / total egg weight.

Total egg weight (g): average total weekly egg weight of each quail in each cage.

Total egg weight = total egg weight per cage per week / number of quails per cage.

Average egg weight (g): total weight of eggs laid by each cage of quails within a week / number of quails

Average egg weight = total weight of eggs per cage per week / total number of eggs per cage per week.

PCR amplification of target fragment

The total volume of reaction systems amplified by PCR was 15μ L. The amplification reaction system comprises: 5.3 μ L of deionized water, 2 × 7.5 μ L of TaqPCR green mix totaling, 0.6 μ L of upstream primer, 0.6 μ L of downstream primer and 1.0 μ L of DNA template. The amplification reaction conditions were: pre denaturation at 95 °C for 5 min, denaturation at 95 °C for 30 s, annealing at 58 °C for 40 s, extension at 72 °C for 40 s, 30 cycles, extension at 72 ° C for 10 min.

PCR-RFLP analysis

The PCR products amplified by ESR-E1 and ESR-E4 primers were digested by restriction enzyme PvuII. The digestion reaction system contained 15 μ L: 5μ L of ddH_2O, 8.5 μ L of PCR product, 0.5 μ L of restriction endonuclease PvuII (10 U· μ L^{-1}), and 1.5 μ L of 10 × buffer. After evenly mixing, they were put it into a 37 °C water bath for digestion for 4 hours. Restriction enzyme AccI is the PCR product amplified by ESR-E8 primer. The enzyme digestion reaction system contained 10 μ L: 0.5μ L of ddH2O, 8 μ L of PCR product, 0.5 μ L of restriction endonuclease PvuII (10 U· μ L^{-1}), and 1. 0 μ L of 10 × buffer. They were also mixed evenly and digested in a 37 °C water bath for 4h. The enzyme products were detected by agarose gel electrophoresis at a 2% concentration. After the gel was drained, the gel imaging system was used to record the photos.

Sequencing and sequence comparison analysis of amplified products

Sequence alignment uses the blast system on the NCBI website to compare the measured gene sequences and confirm whether the sequence is the target sequence through a comparison. The sequencing map was observed and analyzed with the Chromas software to find potential mutation sites, and records and statistics were made.

Association analysis between ESR gene and economic traits

Model: $y_{ijk}=\mu+B_i+M_j+e_{ijk}$

Where y_{ijk} is the phenotypic value of the trait, μ Is the overall mean, B_i is the i-th variety effect (I = 1, 2, 3), M_j is the j-th genotype effect, and e_{ijk} is the residual effect.

RESULTS

PCR product detection of ESR gene

The results of agarose gel electrophoresis for the ESR gene in the PCR products of 3 laying quail populations are shown in Figure 1. As can be seen in Figure 1, the detection results for exon 1 showed only one band, and the fragment size of the detection result is about 370 bp; while the amplified product of exon 4 had a bright band between 250 and 500 bp. The results showed that the fragment size was about 301bp. The amplification results for exon 8 showed a single band, and the size was about 212 bp.

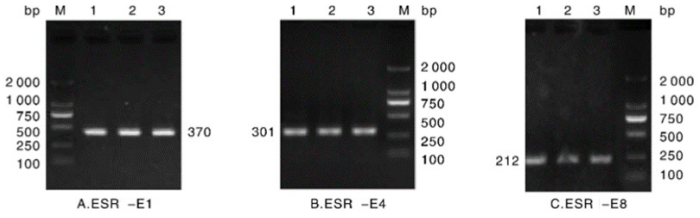

Figure 1 – Detection of PCR product of ESR gene by agarose gel electrophoresis.

Note: M, DL2000 marker; 1. Chinese Yellow Feather Quail; 2. Beijing White Feather Quail; 3. Korean quail.

Polymorphism detection of ESR gene

The results of the PCR-RFLP polymorphism detection for the exon 1 of the ESR gene in the three quail populations of the study are shown in Figure 2. It can be seen that exon 1 of the ESR gene presented the CC (121 bp / 91 bp), CT (212 bp / 121 bp / 91 bp), and TT genotypes (212 bp).

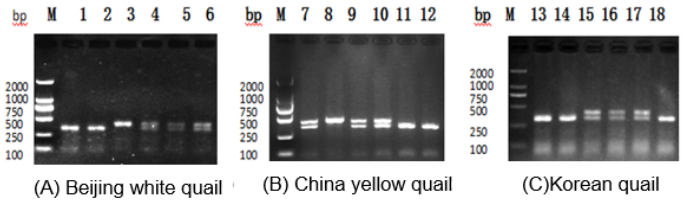

Figure 2 – Polymorphism detection results of exon 1 of ESR gene.

Note: M. DL2000 marker; 3. 8 is TT genotype; 1. 2, 11, 12, 13, 14 and 18 were CC genotypes; 4. 5, 6, 7, 9, 10, 15, 16 and 17 are CT genotypes.

Figure 3 shows the polymorphism results of exon 8 of the ESR gene for the three quail populations. Three genotypes can visibly be detected, namely the CC (121 bp / 91 bp), CT (212 bp / 121 bp / 91 bp) and TT genotypes (212 bp). The two bands of 121 bp and 91 bp are close, and 2% agarose electrophoresis could not distinguish the two bands. Therefore, 121 bp and 91 bp are shown as a relatively thick band in the picture.

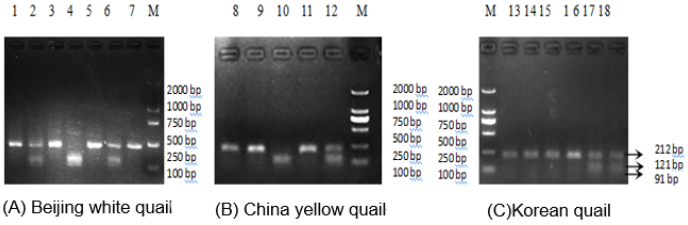

Figure 3 – Polymorphism detection of exon 8 of ESR gene in quails.

Note: M. DL2000 marker, 1, 3, 5, 7, 8, 9, 11, 13, 14, 15 and 16 are TT genotypes, 4 and 10 are CC genotypes, and 2, 6, 12, 17 and 18 are CTDD genotypes.

Sequencing analysis of ESR gene

The sequencing peaks of the SNP sites of exon 4 of ESR gene in three egg quail populations were analyzed using the Chromas software. The correlation analysis results were as follows:

The sequencing results of the PCR amplification products for different genotypes of the exon 1 of the quail ESR gene are shown in Figure 4. C / T mutation was detected at 312 bp in exon 1 of the ESR gene.

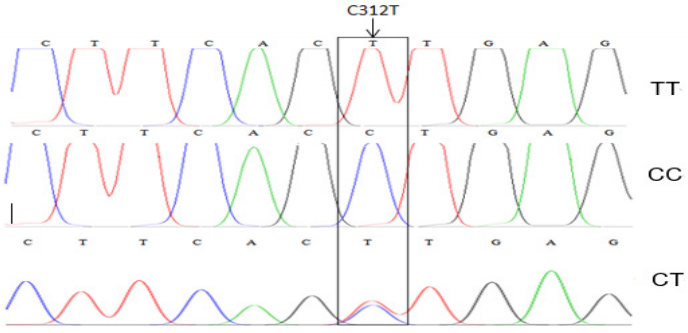

Figure 4 – Sequencing peak map of exon 1 of the ESR gene in quails.

The sequencing results of PCR amplification products for different genotypes of quail ESR gene exon 4 are shown in Figure 5. We can analyze that three genotypes, CC, CT and TT, were detected in three laying quail populations, and the C / T mutation was detected in exon 4 of the ESR gene at 50 bp.

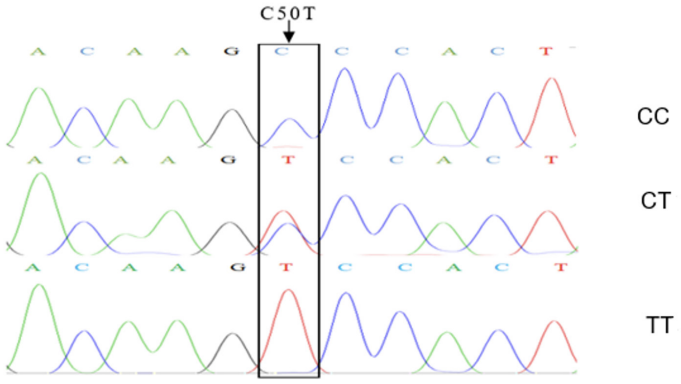

Figure 5 – Sequencing peak map of exon 4 of the ESR gene in quails.

The PCR amplification products of different genotypes of exon 8 of ESR gene were sequenced, as shown in Figure 6. The sequencing results showed that there was a T / C mutation at 91 bp.

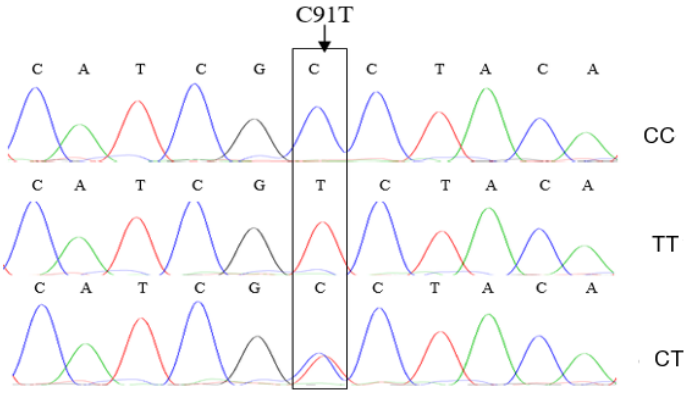

Figure 6 – Sequencing peak map of exon 8 of the ESR gene in quails.

Gene frequency and genotype frequency of ESR gene

It can be seen from Table 2 that the frequency of exon 1 of the ESR gene is the highest among the three egg quail populations. Exon 4 of the ESR gene showed the highest frequency of the TT genotype in Chinese Yellow Feather Quails and Korean quails, and the highest frequency of the CC genotype in Beijing White Feather Quails. The frequency of TT genotype was the highest in exon 8 of ESR gene in the three egg quail populations. Heterozygosity (0.409, 0.491, 0.482), effective allele number (1.693, 1.964, 1.932), and polymorphism information content (0.326, 0.370, 0.366) of exon 1, exon 4 and exon 8 of the ESR gene in Chinese yellow quail population were the highest. We can conclude that Chinese yellow quails have the most abundant genetic polymorphism compared with Beijing white quails and Korean quails.

Table 2 – Population genetic polymorphism of the ESR gene.

Primer Name	Group	Genotype Frequency			Gene Frequency		He	Ne	PIC
		TT	CC	CT	T	C			
ESR-E1	Chinese yellow quail	0.088	0.515	0.397	0.287	0.713	0.409	1.693	0.326
	Beijing white quail	0.014	0.600	0.386	0.207	0.793	0.328	1.489	0.274
	Korean quail	0.015	0.723	0.262	0.146	0.854	0.249	1.332	0.218
ESR-E4	Chinese yellow quail	0.409	0.318	0.273	0.432	0.568	0.491	1.964	0.370
	Beijing white quail	0.098	0.235	0.667	0.784	0.216	0.339	1.512	0.281
	Korean quail	0.617	0.277	0.106	0.245	0.755	0.37	1.587	0.302
ESR-E8	Chinese yellow quail	0.333	0.146	0.521	0.594	0.406	0.482	1.932	0.366
	Beijing white quail	0.708	0.000	0.292	0.854	0.146	0.249	1.332	0.218
	Korean quail	0.500	0.167	0.333	0.667	0.333	0.444	1.799	0.346

Correlation analysis between the ESR gene and egg production performance of quails

The results for the correlation analysis between the ESR gene and quail egg production performance are shown in Figure 7. The results show that exon 1 of ESR gene has no correlation with quail egg production performance (p>0.05). The number, quantity, and rate of layed eggs for the TT genotype were higher than those of the CC and CT genotype. The feed-egg ratio and egg weight of the CC genotype were higher than those of the CT and TT genotypes. The hatch weight of the CC genotype was lower than that of the CT and TT genotypes.

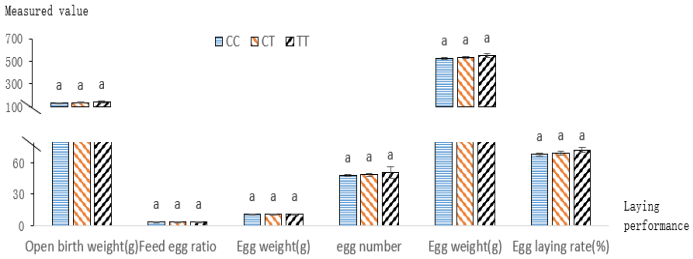

Figure 7 – Correlation analysis of ESR gene exon 1 and quail egg production performance.

Note: lowercase letters indicate significant difference (p<0.05), and lowercase letters mean the difference is not significant (p>0.05).

The results of association analysis between exon 4 of the ESR gene and egg production performance of laying quails are shown in Figure 8. It can be seen from Figure 8 that in the three laying quail populations of exon 4 of the ESR gene, the feed-egg ratio of the CC genotype is significantly higher than that of the CT and TT genotypes (p<0.05), while the feed-egg ratio of the CT genotype is significantly higher than that of the TT genotype (p<0.05). The egg number and egg rate of the TT genotype were significantly higher than those of the CC and CT genotypes (p<0.05), while the egg number and egg rate of the CT genotype were significantly higher than those of the CC genotype (p<0.05). The initial hatch weight of the TT genotype was significantly higher than that of the CC and CT genotypes (p<0.05), but there was no significant difference in initial hatch weight between the CC and CT genotypes (p>0.05). There was no significant difference in egg weight and body weight between the CC, CT and TT genotypes of exon 4 of the ESR gene and the three laying quail populations (p>0.05).

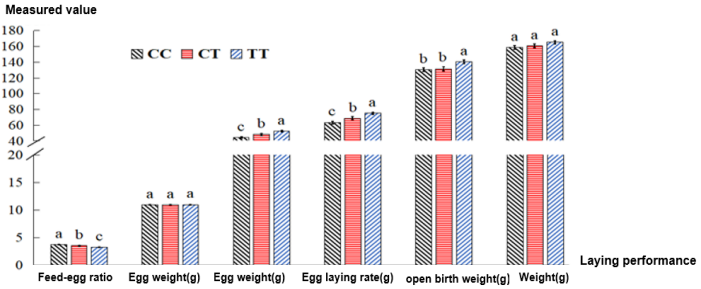

Figure 8 – Correlation analysis of ESR gene exon 4 and quail egg production performance.

Note: lowercase letters indicate significant difference (p<0.05), and lowercase letters mean the difference is not significant (p>0.05).

The correlation analysis between ESR gene exon 8 and quail egg production performance is shown in Figure 9. It shows that the feed-egg ratio of the TT genotype in exon 8 is significantly higher than that of ct9 and CC genotype (p<0.05), the egg number and

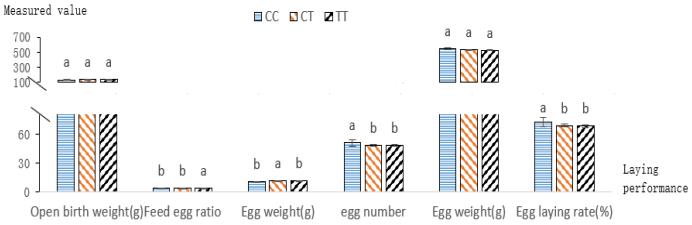

Figure 9 – Correlation analysis of ESR gene exon 8 and quail egg production performance.

Note: lowercase letters indicate significant difference (p<0.05), and lowercase letters mean the difference is not significant (p>0.05).

egg laying rate of CC genotype are significantly higher than that of the CT and TT genotypes ($p<0.05$), and the egg weight of the CT genotype is significantly higher than that of tt9 and CC genotype ($p<0.05$).

DISCUSSION

Analysis of ESR gene polymorphism

In different animal tissues, the content of ESR and its biological functions are also different. The distribution and expression of the ESR gene will also vary according to individual animals and ages. The estrogen receptor gene is not only expressed in animal reproductive systems, but also in animal bones, muscles and livers. In regards to its two sub-types, ESR plays a key role in the reproductive system, while ESR2 mainly affects the central nervous system, immune system, cardiovascular system, urinary system, kidneys, lungs and bones.

When studying the ESR gene in Black Feather Quail β, Xie *et al*. (2016) found that there were single nucleic acid polymorphisms in exons 1, 3, 4, 5, 6 and 8, and the genotype frequency of exon 4 of ESR gene was basically the same. In this study, CC, CT and TT genotypes were detected in the three egg quail populations, and t was also the dominant gene in all three populations. The C50T locus of exon 4 of the ESR gene was moderately polymorphic in Chinese Yellow Feather quails ($0.25 < pic < 0.5$). Zhou *et al*. (2016) found that there are polymorphisms in Exon 1, 3, 4, 6 and 8 of the ESR1 gene. In the egg laying quality of Exon 8, there are significant differences among the CD, DD and CC genotypes. Indicates ESR1 There are indications of a certain correlation in ESR1 between genes and egg laying traits of black feathered laying quails. When exploring the polymorphism of the ESR1 gene and its relationship with quail egg quality, Bai *et al*. (2019) detected the CC, CT and TT genotypes in exon 1, exon 4 and exon 8 of Beijing white quails, Chinese yellow quails and Korean quails, which is consistent with the results of this study. Wang *et al*. (2015) explored that there is a certain correlation between the ESR1 gene and laying traits of Qiandongnan Xiaoxiang chickens. In the breeding process, selecting DD genotype individuals is conducive to improving laying performance without affecting other good traits.

In this study, exon 1, exon 4 and exon 8 of the ESR gene had the highest heterozygosity, number of effective alleles, and polymorphism information content in the Chinese yellow quail population. It can be concluded that Chinese yellow quails have the most genetic polymorphism when compared with Beijing white quails and Korean quails.

Association analysis between ESR gene and reproductive performance

When discussing chicken ESR α, Chen *et al*. (2007) detected two genotypes in the 5 'regulatory region of the gene. Sequencing analysis showed that there were four SNPs, namely A-34G, T-76C, T-79C and C-102T. There was no significant difference in early egg production performance between the two genotypes in Wenchang chickens ($p>0.05$). The results suggest that, in chicken ESR α, four SNPs in the 5 'regulatory region of the gene had no significant effect on egg laying traits. Wu (2013) studied the association between the ESR gene and egg production performance of the Shaobo chicken's maternal line (S2). The results showed that the EE type and EF type were significantly higher than the FF type in terms of egg numbers at 43 weeks of age ($p<0.01$). Zou *et al*. (2019) showed that ESR1 plays an important role in the early gonadal development of Leizhou Black Ducks, and the SNP of ESR1 (g.190744A>G) can be used as an important egg laying marker gene for Leizhou Black Ducks. Li *et al*. (2010) found that in the Landrace population, the total litter size, live litter size, and litter weight of BB individuals of the ESR gene in primiparous sows were higher than those of AA individuals. Yu *et al*. (2008) used PCR-SSCP and PCR-RFLP techniques to detect the single nucleotide polymorphism of the ESR gene in Wenchang Chickens. It was found that there was a certain correlation between various genotypes of the ESR gene and the total number of eggs produced by Wenchang Chicken at 42 weeks of age ($p<0.05$). Tang *et al*. (2009) also showed that the polymorphism of the ESR gene was significantly correlated with the number of eggs laid by Wenchang Chickens at 42 weeks of age ($p<0.05$), and also with the average number of days of continuous laying ($p<0.01$). Ji *et al*. (2013) found that exon 4 of the ESR gene in Dongxiang green shell laying hens was significantly correlated with the average egg production at 300 days of age ($p<0.05$). Wang *et al*. (2012) found that there was significant difference between ESR gene, feed-egg ratio and egg number for Zhuanghe big bone chickens ($p<0.05$). Qin *et al*. (2017) found that the ESR α AB genotype for the A-119854T and C-158689G loci of the gene had a significant effect on the egg numbers of 300-day-old rose crown chickens ($p<0.05$), which is expected to be used as a molecular marker related to the egg production performance of rose crown chickens. Zhou *et al*. (2016) found that exon 1 of the ESR gene had a significant effect on

the starting laying age of black feather egg quails (p<0.05), while exon 8 had a significant correlation with egg weight at the beginning of laying (p<0.05). Li *et al*. (2021) found that there was a significant relationship between h5h5 doubling and egg shape index of white shell laying hens (p<0.05). In this study, the association analysis between exon 1, exon 4 and exon 8 of the ESR gene and egg production performance of three laying quails was also carried out. The results showed that in exon 1 of the ESR gene, the number, quantity and rate of laying eggs of the TT genotype were higher than those of the CC and CT genotypes. The feed-egg ratio and egg weight of the CC genotype were higher than those of the CT and TT genotypes. The starting weight of the CC genotype was lower than that of the CT and TT genotypes, but there was no significant difference among the three genotypes, indicating that exon 1 of the ESR gene was not associated with the egg production performance of quails (p>0.05). In exon 4 of the ESR gene, the feed-egg ratio of the CC genotype was significantly higher than that of the CT and TT genotypes (p<0.05). The TT genotype was significantly higher than the CC and CT genotypes in egg number, egg laying rate and starting weight (p<0.05), and there were significant differences among the three genotypes, indicating that exon 4 of the ESR gene was significantly associated with the egg production performance of quails (p<0.05). In exon 8 of the ESR gene, the feed-egg ratio of the TT genotype was significantly higher than that of the CC and CT genotypes (p<0.05), the egg number and egg laying rate of the CC genotype were significantly higher than that of the CT and TT genotypes (p<0.05), and the egg weight of the CT genotype was significantly higher than that of the TT and CC genotypes (p<0.05). There were significant differences among the three genotypes, indicating that exon 8 of the ESR gene was significantly correlated with the egg production performance of quails (p<0.05).

CONCLUSIONS

By detecting the polymorphisms of exon 1, exon 4 and exon 8 of the ESR gene in three laying quail populations, and analyzing the association with quail egg production performances, we conclude that the ESR gene can be used as a candidate gene for molecular marker assisted selection of egg production performance of laying quails. This provides a theoretical basis for cultivating new laying quail lines with better egg production performances.

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