



Short Communication

Correlation Analysis of Exon 4 of Estrogen Receptor Gene with Growth Traits of Quail (*Coturnix coturnix*)

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ABSTRACT

The purpose of this study was to investigate the effect of estrogen receptor gene (*ESR*) polymorphism on the growth traits of quails. PCR products were used to directly sequence the polymorphism of exon 4 of *ESR* gene in three quail populations (Chinese yellow quail, Korean quail, Beijing white quail) and analyze the association between *ESR* gene and growth traits of quails. The results show that three genotypes, CC, CT and TT, were detected in exon 4 of *ESR1* gene in three quail populations. The highest frequencies of TT gene were 0.409 and 0.617 in China yellow quail and Korean quail, respectively. The highest frequencies of CC gene were 0.667 in Beijing white quail. For exon 4 of *ESR1* gene in China yellow quail ($P<0.05$), the weight and tibial length of the TT genotype were significantly higher than the CT and CC genotype ($P<0.05$). Among Beijing white quail, the weight of CC genotype was significantly higher than that of CT and TT genotypes ($P<0.05$), and the sternum length of CC genotype was significantly higher than that of TT genotype ($P<0.05$). Among Korean quail, the body weight of CT genotype was significantly higher than that of CT and TT genotype ($P<0.05$), and the tibial length of CT genotype was significantly higher than that of TT genotype ($P<0.05$). It was found that exon 4 of *ESR1* gene was significantly associated with body weight, tibial length and sternum length of egg quail ($P<0.05$).

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Authors' Contribution

JYB conceived and designed the study and conducted the lab work. YC and QHH analyzed the data and wrote the article. ZHD, YZP and YBY helped in sampling. YL and SJZ helped in analysis of data.

Key words

Coturnix coturnix, Estrogen receptor gene, Polymorphism analysis, Correlation analysis, Growth traits

Quail egg also contains high levels of lecithin, cephalin, vitamins and iron. Quail meat and eggs also have certain therapeutic effects on asthma, tuberculosis, metabolic disorders, neurasthenia and other diseases, and have a relatively high medicinal value. Since, there are many factors affecting the production and reproduction performance of quail, and the genetic ability of quail is low, the progress of conventional breeding is slow. At present, quail breeding is becoming more and more popular in poultry, and since it is smaller than other poultry, it can be used as a new experimental animal for studies on poultry reproduction, nutrition, physiology, pharmacology and so on (Li *et al.*, 2019; Bai *et al.*, 2013, 2016, 2017). Several researchers have studied estrogen receptor gene (*ESR*) in mammals and poultry (Zhang *et al.*, 2013; Zhou *et al.*, 2008; Si *et al.*, 2016), but the correlation analysis between *ESR* gene and growth traits have not been reported. Therefore, in order to provide a reference for marker-assisted selection of quails, the association of exon 4 of

ESR gene with growth traits was analyzed by using sequencing technology with quail as experimental animals.

Materials and methods

In this experiment, blood samples (5ml) of 50 China quails with yellow feathers, 50 Beijing quails with white feathers and 50 Korean quails, all females, were collected from veins in wings and stored in heparin sodium anticoagulant tubes which were then kept in a refrigerator under -20°C . DNA was extracted by poultry whole blood DNA kit and kept at -20°C .

Primers of exon 4 of *ESR1* gene were designed according to Pu (2016). The primer sequences are as follows: F: CGGGCGAATGATGAAACA, R: CCCAGTTGATCATGTGCA. The size is 301bp. Primers were synthesized by Beijing Dongguo Changsheng Biotechnology Co., Ltd. The thermal cycle comprised pre-denaturation at 94°C for 4 min, then 35 cycles each of denaturation at 94°C for 40s, annealing at 58°C for 1 min and elongation at 72°C for 20 seconds, followed by extension at 72°C for 10 min. PCR products were sent to Zhengzhou Dingguo Biology Co., Ltd. for nucleotide sequencing.

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SPSS17.0 statistical software was used to analyze the association between different genotypes and growth traits, and Duncan multiple comparison method was used to make multiple comparison. The final results were expressed in the form of mean value \pm standard error.

Results and discussion

Figure 1 shows the PCR product of exon 4 of *ESR* gene. Figure 2 shows sequencing results exon 4 of *ESR* gene showing one SNP site at C50T and three genotypes.

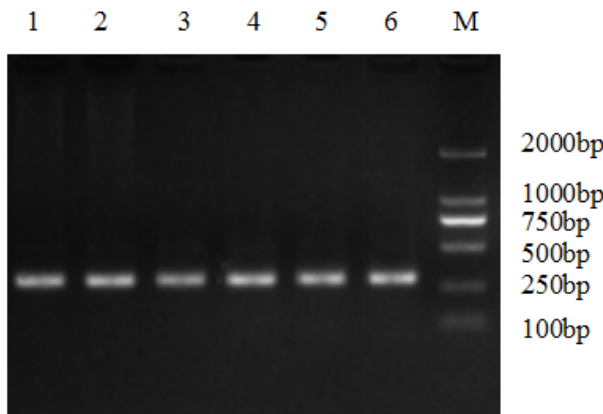


Fig. 1. Detection of PCR products of exon 4 of *ESR* gene. Note: m is DL2000 marker, 1-2 are Chinese yellow quail, 3-4 are Beijing white quail, 5-6 are Korean quail.

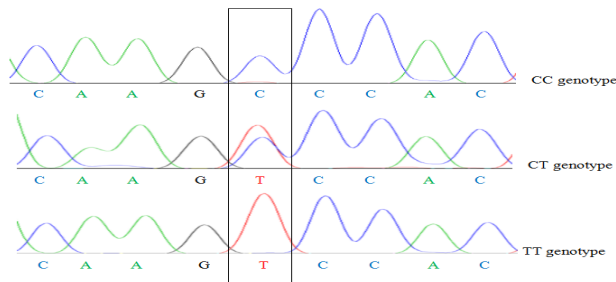


Fig. 2. Detection of SNP locus in exon 4 of *ESR* gene.

The results of population genetic polymorphism of exon 4 of *ESR* gene are shown in Table I. Three genotypes, CC, CT and TT, were detected in exon 4 of *ESR* gene in three quail populations. The highest frequencies of TT gene were 0.409 and 0.617 in China yellow quail and Korean quail, respectively. The highest frequencies of CC gene were 0.667 in Beijing white quail. The frequencies of exon 4 of *ESR* gene of T allele in China yellow quail and Korean quail were the highest (0.568, 0.755, respectively). The frequencies of exon 4 of *ESR* gene of C allele in Beijing white quail was as high as 0.784. The effective allele

number of exon 4 of *ESR* gene in three quail populations was 1.512-1.964, and the polymorphic information content was 0.281-0.370. The highest heterozygosity of China yellow quail was 0.491, and the lowest heterozygosity of Korean quail was 0.339. A single SNP mutation site was detected in exon 4 of *ESR* gene. The exon 4 in Chinese yellow and Korean quail groups generated respectively a higher frequency of T allele than C allele, which made the T allele the dominant allele in these populations. This finding was similar to the results of Pu (2016), although the dominant allele being C allele among the Chinese yellow quails went opposite to Pu's findings.

The results of correlation analysis between the polymorphism of exon 4 of *ESR* gene and the growth traits of quail are shown in Table II. For exon 4 of *ESR* gene in China yellow quail, the weight and tibial length of the TT genotype were significantly higher than the CT and CC genotype ($P < 0.05$). Among Beijing white quail, the weight of CC genotype was significantly higher than that of CT and TT genotypes, and the sternum length of CC genotype was significantly higher than that of TT genotype ($P < 0.05$). Among Korean quail, the body weight of CT genotype was significantly higher than that of CT and TT genotype, and the tibial length length of CT genotype was significantly higher than that of TT genotype ($P < 0.05$). It was found that exon 4 of *ESR* gene was significantly associated with body weight, tibial length and sternum length of egg quail ($P < 0.05$).

Only a few studies were related with the *ESR* gene and growth performance of livestock and poultry. Wang *et al.* (2009) and others indicated in their studies that AA genotypes of *ESR* gene produced greater body length, and more significant body height, chest circumference and body weight of Su Jiang pigs than BB genotypes. Liu (2007) found that *ESR* gene had obvious influences on the daily gain of Jiangxi and Anhui large white pigs, but showed little effects on their body height, body length and chest circumference. Li (2015) however suggested that there was no significant difference in early growth traits among Suhuai pigs with various genotypes of *ESR* gene. The results of this study are similar to those of Wang *et al.* (2009) but inconsistent with those of Liu (2007) and Li (2015). In this paper, it was found that exon 4 was correlated with the body weight, shank length and breastbone length. Although Korean, Beijing white and Chinese yellow populations are egg quails, in addition to egg yield, the body weight, length of shank, breastbone and the body, and shank circumference shall be given considerations to when in breeding. As a result, exon 4 of *ESR* gene may be used as candidate genes for marker-assisted selection of egg quails' growth traits.

Table I. Gene genotype frequency and allele frequency of *ESR* gene.

Group	Genotype frequency			Allele frequency		Hybridiza- tion (He)	Number of effec- tive alleles (Ne)	Polymorphic informa- tion content (PIC)
	CC	CT	TT	C	T			
Chinese yellow quail	0.273	0.318	0.409	0.432	0.568	0.491	1.964	0.370
Beijing white quail	0.667	0.235	0.098	0.784	0.216	0.339	1.512	0.281
Korean quail	0.106	0.277	0.617	0.245	0.755	0.370	1.587	0.302

Table II. Correlation analysis of *ESR* gene exon 4 and 9-15 weeks growth traits of quail.

Varieties	Genotype	Weight (g)	Tibia length (cm)	Chest width (cm)	Chest depth (cm)	Breastbone length (cm)	Body length (cm)	Tibia circum- ference (cm)
Chinese yellow quail	CC	161.049±1.849 ^b	3.607±0.016 ^a	3.187±0.019 ^a	3.344±0.026 ^a	4.474±0.024 ^b	9.483±0.052 ^a	1.696±0.013 ^a
	CT	163.719±1.903 ^b	3.619±0.015 ^a	3.193±0.020 ^a	3.351±0.023 ^a	4.468±0.027 ^b	9.409±0.056 ^a	1.683±0.012 ^a
	TT	168.415±1.337 ^a	3.616±0.011 ^a	3.234±0.016 ^a	3.359±0.018 ^a	4.540±0.018 ^a	9.508±0.043 ^a	1.708±0.010 ^a
Beijing white quail	CC	155.027±1.094 ^a	3.541±0.009 ^a	3.067±0.013 ^a	3.185±0.015 ^a	4.291±0.012 ^a	8.872±0.034 ^a	1.644±0.007 ^a
	CT	150.873±1.717 ^a	3.522±0.016 ^a	3.051±0.022 ^a	3.219±0.024 ^a	4.248±0.018 ^{ab}	8.875±0.057 ^a	1.620±0.011 ^a
	TT	141.629±5.884 ^b	3.505±0.029 ^a	3.097±0.036 ^a	3.136±0.061 ^a	4.207±0.052 ^b	8.964±0.175 ^a	1.629±0.024 ^a
Korean quail	CC	183.954±2.594 ^a	3.728±0.022 ^a	3.303±0.031 ^a	3.422±0.043 ^a	4.360±0.033 ^a	9.334±0.073 ^a	1.714±0.021 ^a
	CT	173.976±1.296 ^b	3.671±0.015 ^{ab}	3.251±0.018 ^a	3.436±0.026 ^a	4.298±0.020 ^a	9.296±0.040 ^a	1.711±0.009 ^a
	TT	172.757±1.359 ^b	3.668±0.011 ^b	3.256±0.013 ^a	3.429±0.017 ^a	4.329±0.014 ^a	9.335±0.030 ^a	1.709±0.008 ^a

Note: If there are different lowercase letters in different columns of the same variety, the difference is significant ($P < 0.05$), if there are same lowercase letters, the difference is not significant ($P > 0.05$).

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Statement of conflict of interest

The authors have declared no conflict of interest.

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