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SNP analysis reveals estrogen receptor 1 (*ESR1*) gene variants associated with laying traits in quails

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Abstract. In this study, the estrogen receptor 1 (*ESR1*) gene was studied as a candidate gene for laying traits of two quail populations (the yellow-feather quail and chestnut-feather quail). Five pairs of primers were designed to detect single-nucleotide polymorphisms (SNPs) of exon 1, 2, 4, 8 and intron 1 of the *ESR1* gene by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) and sequencing methods. Only the products amplified from exon 8 displayed polymorphism. The results showed one novel variation: a variation in exon 8 of *ESR1* gene (g.91C > T, KC977991 and KC977992). It was associated with some laying traits in two quail populations including egg weight, the age of first egg and egg number at 20 weeks. And the CC genotype was associated with superior egg number at 20 weeks. Therefore, we speculated that the variation in exon 8 of *ESR1* gene may have an effect on laying traits in the abovementioned quail populations.

1 Introduction

Laying performance is an economically important trait in egg-producing birds and is always of primary concern during breeding. This has led commercial breeders to incorporate significant selection for increased laying performances in breeding programmers. The quest for improved egg production is an important focus of poultry breeding and management (Kang et al., 2009). Estrogen is known to be involved in the regulation of oogenesis, vitellogenesis, gonadotropin regulation, testicular development and other aspects of reproduction, in addition to having regulatory roles in many organ systems (Gustafsson, 2003; Heldring et al., 2007; Hess et al., 2003; Muriach et al., 2008; Nilsson et al., 2001). The biological actions of estrogens are manifested through two high-affinity estrogen receptors (*ESRs*), estrogen receptor alpha and estrogen receptor beta (Gruber et al., 2004; Kuiper et al., 1996). *ESRs* belong to the nuclear receptor superfamily, a family containing receptors for small molecules (steroids, thyroid hormones, rexinoids, oxysterols, etc.) with a defined domain structure (Nelson and Habibi, 2013). *ESRs* are also thought to play a central role in the regulation of many life processes, including development, reproduction and normal physiology (Böttner et al., 2014). The role of *ESR1* and *ESR2* in the reproductive performance of poultry has been increasingly studied in recent years (Tang et al., 2009; Wang et al., 2012). *ESR1* gene is one of the candidate genes for detecting polymorphisms associated with the reproductive and laying traits in pigs, chickens and other animals (Goliasova and Wolf, 2004; Humpolicek et al., 2007; Liu et al., 2010; Muñoz et al., 2007; Tang et al., 2009; Wang et al., 2012; Bi et al., 2005; Szreder and Zwierzchowski, 2007; Wu et al., 2014). The objectives of this study were to detect the polymorphism in five regions (exons 1, 2, 4, 8 and intron 1) of *ESR* gene in quail populations (the yellow-feather and chestnut-feather quails), which are bred in China and have relatively high laying performance and mature early (about 45 days), and to investigate its association with laying traits of two populations and further search the possibility of *ESR1* gene being used as molecular genetic markers for laying traits.

Table 1. Primer sequence, product size and annealing temperature used in analyses of quail *ESR* gene.

Primer name	Product size (bp)	Primer sequence (5′–3′)	Amplified region	Annealing temperature (°C)
P1	382	F: CAAAGCCTCTGGAGTTAC R: CAAAGCTGCCCTGTTCAT	Exon 1	54.1
P2	161	F: CAAGCTCTGATAATAGGCG R: AGCCCTCACAAGACCAGAC	Exon 2	58.8
P3	303	F: GCGGGCGAATGATGAAACA R: GCCCAGTTGATCATGTGCA	Exon 4	57.6
P4	213	F: CAACAAAGGAATGGAGCA R: CCTCTTCTTTGCTGTTAA	Exon 8	53.6
P5	161	F: CAAGCTCTGATAATAGGCG R: AGCCCTCACAAGACCAGAC	Intron 1	58.0

2 Materials and methods

2.1 Sample collection

Two quail populations (the yellow-feather quails, 192, and the chestnut-feather quails, 192) were used in this study for initial single-nucleotide polymorphism (SNP) discovery. The two populations were raised on the same farm and selected for 10 years for different feather color and laying performance. The yellow-feather quail has higher egg weight, and the chestnut-feather quail has higher egg number. All birds were raised under identical environmental conditions: in cages and fed commercial corn–soybean diets that met quails' requirements. Blood samples (collected from 14th generation) and phenotypic data on laying traits (the weight of first egg, the age of first egg, egg weight, the weight at 20 weeks and the egg number at 20 weeks) were collected from the 384 individuals. Genomic DNA was obtained by phenol and chloroform (1 : 1) extraction, and stored at −20 °C.

2.2 Primer design and PCR amplification

According to chicken *ESR1* gene sequences (GenBank accession no. NC_006090), five pairs of primers (Table 1) were designed to amplify the quail *ESR1* gene exon 1, 2, 4, 8 and intron 1 regions and detect the SNP of the five regions.

The polymerase chain reaction (PCR) was performed by mixing 40 ng of genomic DNA, 0.5 pmol of each of forward and reverse primer, 1.5 µL of 10 × buffer, 1.5 mM of $MgCl_2$, 0.25 mM of each dNTP, and 1.5 U of *Taq* DNA polymerase (Fermentas, Shenzhen, China) in a 15 µL volume, and run on an Eppendorf Mastercycler gradient (Eppendorf, Hamburg, Germany) according to the following program: 95 °C for 5 min; 35 cycles of 94 °C for 30 s, annealing of X °C (Table 1) for 35 s, and 72 °C for 45 s; and final extension step at 72 °C for 10 min.

2.3 SNP identification and genotyping

Genomic DNA from the yellow-feather quails and chestnut-feather quails was used as a template to amplify with the five pairs of primers (exon 1, 2, 4, 8 and intron 1), and the sequences were aligned to search for base variations.

The polymorphisms in quail *ESR1* were analyzed by PCR-RFLP (restriction fragment length polymorphism), which was performed by mixing 8 µL of PCR product (with primer exon 8), 10 U of the restriction enzyme *Acc*I (Fermentas, Shenzhen, China) and 1 µL corresponding 10 × reaction buffer, and incubated at 37 °C for 12 h, and then separated at 125 V on a 3 % agarose gel.

2.4 Statistical analysis

The genotypic frequencies were calculated, and the Hardy–Weinberg equilibrium for each breed was analyzed using χ^2 test of PopGene32 (version 1.31). The traits were compared among the genotypes. The association of *ESR1* genotypes with laying traits including the weight of first egg, the age of first egg, egg weight, the weight at 20 weeks and the egg number at 20 weeks were evaluated according to two-way analysis of software SPSS (version 16.0), using the following model:

$$Y = \mu + G + L + G \times L + e,$$

where Y is the dependent variable (analyzed traits); μ was the overall mean; genotype (G) of *ESR1* exon 8 (CC, CT and TT), the quail population (L), interactions between genotype and quail population ($G \times L$) were the fixed effects; and e was the random error. Difference between genotypes was determined by least squares.

3 Results

3.1 Polymorphism identification and detection

The sequences amplified with five pairs of primers (P1, P2, P3, P4 and P5 for exon 1, 2, 4, 8 and intron 1) were aligned

Table 2. Allele and genotype frequencies at exon 8 locus of *ESR* gene in two quail populations.

Breeds	Number	Genotype frequencies			Gene frequencies		χ^2 (HWE)
		CC	TT	TC	C	T	
Yellow-feather quail	192	0.5625	0.0208	0.4167	0.7708	0.2292	6.1018*
Chestnut-feather quail	192	0.5625	0.0625	0.3750	0.7500	0.2500	1.3932

Note: $\chi 2$ (HWE): Hardy–Weinberg equilibrium $\chi 2$ value; * showed that $P<0.01$ and the SNP locus in the population was not at Hardy–Weinberg equilibrium.

Table 3. Least square means and standard errors of the laying traits in two quail populations.

Traits	Yellow-feather quail (mean ± SE)			
	CC ($n=96$)	TT ($n=28$)	CT ($n=68$)	P value
The weight of first egg (g)	137.58 ± 2.39	139.28 ± 4.17	139.82 ± 2.78	0.819
The age of first egg (d)	44.96 ± 0.76 ^{b}	47.43 ± 1.68 ^{ab}	49.41 ± 0.85 ^{a}	0.007
Egg weight (g)	10.74 ± 0.20	10.78 ± 0.27	10.90 ± 0.28	0.880
The weight at 20 weeks (g)	155.95 ± 2.78	160.00 ± 4.93	157.00 ± 2.72	0.762
The egg number at 20 weeks	86.33 ± 1.39 ^{a}	81.86 ± 0.86 ^{a}	73.94 ± 1.70 ^{b}	0.001
Traits	Chestnut-feather quail (mean ± SE)			
	CC ($n=96$)	TT ($n=16$)	CT ($n=80$)	P value
The weight of first egg (g)	143.54 ± 1.43	144.25 ± 2.28	140.05 ± 2.40	0.390
The age of first egg (d)	45.33 ± 0.53	45.00 ± 1.29	45.05 ± 0.75	0.942
Egg weight (g)	9.83 ± 0.44 ^{a}	8.08 ± 0.62 ^{b}	9.82 ± 0.53 ^{a}	0.006
The weight at 20 weeks (g)	153.58 ± 2.26	153.25 ± 2.28	148.55 ± 2.69	0.419
The egg number at 20 weeks	88.13 ± 1.28^{a}	84.25 ± 1.63^{ab}	82.90 ± 0.98 ^{b}	0.009

Note: different superscript letters (a and b) were significantly different (least significant difference test, $P<0.05$) in genotypes CC, TT and CT; n was the number of the genotype in the population.

among yellow-feather quails and chestnut-feather quails. No base variation was found in exon 1, 2, 4 and intron 1. There was one variation locus in exon 8, C→T at the nucleotide position 91 (g.91C > T), which was synonymous and did not result in an amino acid change. But this variation locus changed the recognition site of restriction endonucleases *Acc*I. This polymorphism can be detected by PCR-RFLP using the amplification product of primer P4. The sequences having variation in exon 8 were submitted to GenBank (accession number: KC977991, for CC genotype, and KC977992, for TT genotype). As shown in Fig. 1, *C* to *T* variation at locus 91 expressed three genotypes: CC, CT and TT.

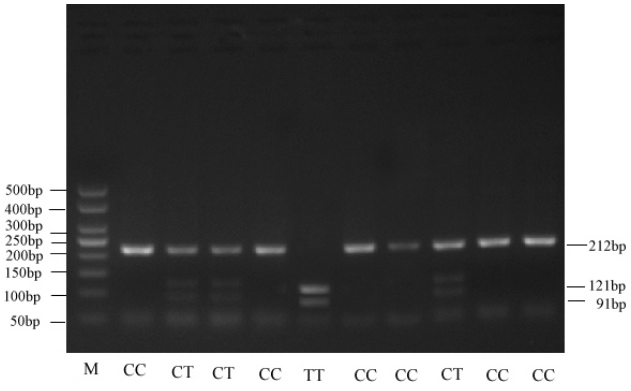

Figure 1. PCR-RFLP band pattern on a 3 % agarose gel. The TT genotype has two bands (121 and 91 bp) which run fast; the CC genotype has one band (212 bp) which runs slowly; the CT genotype has three bands (212, 121 and 91 bp), with one running slowly and the others running fast.

3.2 Allele and genotype distribution of *ESR1* gene exon 8

Allele and genotype frequencies of *ESR1* gene exon 8 in two quail populations are listed in Table 2. The results indicate that at 91 loci, *C* allele is preponderant allele in yellow-feather quail and chestnut-feather quail. The yellow-feather quail population deviated from Hardy–Weinberg equilibrium ($P<0.01$), and the chestnut-feather quail population was in accordance with Hardy–Weinberg equilibrium ($P>0.05$).

3.3 Association of polymorphism with laying traits at *ESR1* gene exon 8

The results of association analysis between different genotypes and laying traits of two quail populations are given in Table 3.

For chestnut-feather quails, there were significant differences between genotype TT and CC or CT for egg weight and between genotype CC and CT for the egg number at 20 weeks ($P<0.05$). For yellow-feather quail, there were significant differences between genotype CC and CT for the age of first egg and between genotype CC or TT and CT for the egg number at 20 weeks ($P<0.05$). Furthermore, no significant association of different genotypes with other traits were detected ($P>0.05$).

4 Discussions

In this study, one variation in exon 8 of *ESR1* gene was discovered. The analyzed results suggested significant association of the exon 8 of *ESR1* gene variation in determining quails' egg weight, the age of first egg and egg number at 20 weeks. And the CC genotype was associated with superior egg number at 20 weeks. Moreover, further birds or other quail breeds need to be tested in order to validate both the associations found and the physiological significance of the variation. Considered overall, we speculated the variation in exon 8 of ESR1 gene may be having an effect on laying traits and could be used as a molecular marker to carry out selective breeding for quails.

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