# Polymorphisms of Vasoactive Intestinal Peptide Receptor-1 Gene and Their Genetic Effects on Broodiness in Chickens

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**ABSTRACT** Broodiness is a polygenic trait controlled by a small number of autosomal genes. Vasoactive intestinal peptide receptor-1 (*VIPR-1*) gene could be a candidate of chicken broodiness, and its genomic variations and genetic effects on chicken broodiness traits were analyzed in this study. The partial cloning and sequencing of the *VIPR-1* gene showed that the average nucleotide diversity was 0.00669 ± 0.00093 in Red Jungle Fowls (RJF), and 0.00582 ± 0.00026 in domestic chickens. One hundred twenty-eight variation sites were identified in the 11,136-bp region of the chicken *VIPR-1* gene. Twenty variation sites were genotyped using PCR-RFLP or PCR method to analyze average diversity, linkage-disequilibrium pattern, and haplotype structure in RJF, Xinghua chickens, Ningdu Sanhuang chickens, Baier Huang chickens, and Leghorn Layers. The RJF, Xinghua, Ningdu Sanhuang, and Baier Huang exhibited distinct characteristic of decreasing $r^2$ value over physical distance. Haplotype analyses showed that some variation sites of the 27-kb region from exon 6 to exon 11 could be associated with broodiness. The distribution of genotypic and allelic frequencies, and heterozygosities in the above 5 populations showed that A–284G, A+457G, C+598T, D+19820I, C+37454T, C+42913T, and C+53327T might be associated with broodiness. The 7 sites and the other 4 sites were genotyped in 644 NDH individuals under cage condition and were used for association analyses between each site and chicken broodiness traits. A significant association ($P < 0.05$) was found between C+598T in intron 2 and broody frequency (%). Another significant association ($P < 0.05$) was found between C+53327T and duration of broodiness, in which allele C was positive for DB.

**Key words**: chicken *VIPR-1* gene, linkage disequilibrium, genetic diversity, broodiness

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## INTRODUCTION

Broodiness is a polygenic trait controlled by a small number of autosomal genes (Romanov et al., 2002). Recent studies had demonstrated that broodiness was not controlled by genes on the Z chromosome (Romanov et al., 2002; Jiang et al., 2005). Although the incidence of broodiness in poultry can be effectively controlled by traditional breeding methods, its eradication is a slow process. With methods of molecular biology and development of genome research, genetics of poultry incubation behavior was studied at the molecular level recently.

To determine whether there are QTL associated with the nonbroody/broody phenotypes, Sharp (2004) genotyped 156 microsatellite markers spaced across the chicken genome in White Leghorn × Silkie cross. However, no major QTL for the broody trait were identified using such genome-wide scan approach. Because prolactin (**PRL**) plays an important role in incubation behavior of avians (Sharp et al., 1984; El Halawani et al., 1993), *PRL* was thought to be an important candidate gene for broodiness. Previous studies showed that a 24-bp indel in the chicken *PRL* promoter region was associated with broodiness (Jiang et al., 2005; Liang et al., 2006). In the past decade, almost all studies were focused on association of the *PRL* gene variation with broodiness, and few other genes were studied.

Vasoactive intestinal peptide (**VIP**) is a PRL-releasing factor in birds (El Halawani et al., 1990, 1997). Passive immunization with VIP antibodies terminated incubation behavior and reduced circulating PRL in incubating chickens (Sharp et al., 1989). Active immunization of turkey hens against VIP resulted in inhibiting incubation behavior and increasing egg production (El Halawani et al., 1995, 2000). The effects of VIP are mediated through interaction with its specific receptor locating on the surface membranes of anterior pituitary cells in avian. The vasoactive intestinal peptide receptor-1 (**VIPR-1**) belongs to the class II subfamily of the 7-transmembrane G-protein-coupled


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receptors superfamily (Gaudin et al., 1998). Like other family members, the VIPR-1 is a glycoprotein with a large hydrophilic extracellular N-terminus followed by 7 highly conserved hydrophobic transmembrane helices and a cytoplasmic C-terminus. The *VIPR-1* gene was proved to be involved in the regulation of broodiness in avian with evidence of endocrine mechanism of broodiness and expression in vivo and in vitro (Rozenboim and El Halawani, 1993; Kansaku et al., 2001; You et al., 2001; Chaiseha et al., 2004). The *VIPR-1* gene was expressed throughout the hypothalamus and pituitary, major in pituitary, and only the differential mRNA expression of the *VIPR-1* gene in the pituitary was associated with reproductive changes (Kansaku et al., 2001; You et al., 2001; Chaiseha et al., 2004). These results suggested that the *VIPR-1* gene might be associated with broodiness. In the present study, the *VIPR-1* gene was selected to analyze its genetic effects on chicken broodiness traits as a candidate. Recently, avian *VIPR-1* genes were cloned and functionally characterized in the chicken (Kansaku et al., 2001) and turkey (You et al., 2001). The chicken *VIPR-1* gene, located on p3.2 of chromosome 2 (Kansaku et al., 2001), spans 67,906 bp and is composed of 13 exons ranging in size from 45 to 1,031 bp.

Domestication and breeding improvement are involved in the selection for specific alleles at genes controlling key morphological and desirable traits, resulting in reduced genetic diversity and increased linkage disequilibrium (**LD**) relative to unselected genes (Yamasaki et al., 2005). It is not proven whether the chicken *VIPR-1* gene endures stronger selection pressure during domestication and genetic improvement. The selection pressure on the *VIPR-1* gene can be tested by studying genetic diversity of the *VIPR-1* gene in chicken populations with different broodiness. Although previous studies had proven that the expression of the *VIPR-1* gene in the transcriptional level was positively correlated with broodiness in chicken and turkey (Kansaku et al., 2001; You et al., 2001), information on DNA variation effects on broody behavior is lacking.

The objective of the present study was to identify the *VIPR-1* gene variations associated with chicken broodiness based on population genetics study and marker-trait association analyses in native population. In this study, polymorphisms of the chicken *VIPR-1* gene were identified by cloning and sequencing method from the chickens with large phenotypic differences in broodiness. Genetic diversity of the chicken *VIPR-1* gene was observed based on some of the above polymorphisms, and the association of the *VIPR-1* gene variations with chicken broodiness was analyzed.

## MATERIALS AND METHODS

### Chicken Populations

Six chicken populations, Red Jungle Fowls (**RJF**), Taihe Silkie (**TS**), Xinghua chickens (**XH**), Gushi chickens (**GS**), White Recessive Rock broilers (**WRR**), and Leghorn Layers (**LH**) were used for identification of the *VIPR-1* gene polymorphisms and average nucleotide diversity. The samples used for polymorphism identification included 24 individuals, 4 from each of the 6 populations.

A total of 364 birds from RJF (33), XH (97), Ningdu Sanhuang chickens (**NDH**, 96), Baier Huang chickens (**BEH**, 41), and LH (97), were randomly sampled and genotyped for average diversity, LD pattern, and haplotype structure analyses. The detailed information of the samples was showed in Table 1. The XH, NDH, and WRR were from Guangdong Wens Foodstuff Corporation Ltd. (Guangdong, China), LH was from Likang Poultry Farm (Guangdong, China).

A NDH population composed of 644 individuals was used for association analyses.

### Observation for Broodiness Traits

All NDH female birds were placed into individual laying cages at 90 d of age, and their incubation behavior from 90 d to 300 d of age was observed and recorded at 1600 to 1700 h. All birds were pedigree wing-banded at hatch and were fed ad libitum to 77 d of age with 16.5% CP and 2,800 kcal of ME/kg, and then fed a corn-soy-bean-based diet with 15.0% CP and 2,900 kcal of ME/kg. All individuals were exposed to a continuous 24-h photoperiod during the first 2 d posthatch, and then changed to 16-h photoperiod, being maintained until 300 d of age.

Broody behaviors were recognized with the following aspects. Hens were found nesting a cage when the cage was being gently beaten. Hens exhibited stress, feather loosening, and lifting, shivering when they were gently beaten. The body temperature of hens increased. Hens showed aggressive or defensive behaviors, specific clucking, turning and retrieval of eggs, and lost their appetite. Hens also showed heavy, prominent, overhanging eyebrows, and lacked of luster throughout the head, comb, and wattles. The face of hens was wrinkled, and the features throughout are more or less masculine. Their reproductive organs were reduced in size, and the vent and whole abdominal region had a tendency to contract. The vent might be fairly large, but it became dry and was surrounded with prominent wrinkles. During the observed period, we found that broodiness was categorized into 2 types, typical broody and discontinuous broody. Typical broody was defined as hens with obvious consecutive broody behavior for more than 3 d and terminative broody days for more than 1 wk. Discontinuous broody was defined as hens exhibiting obvious broody behavior for less than 3 d and terminative broody days for less than 1 wk. Considering enough sample numbers in statistics, all hens with obvious consecutive broody behavior and discontinuous broody were considered as broodiness. The criterion for the end of broodiness was that the hen did not exhibit any broody behavior.

Broodiness traits were assigned to the 2 parameters, duration of broodiness (**DB**) and broody frequency (%). The DB was obtained by counting all the days that a hen became broody during the observation period. Broody frequency (%) was expressed as the percentage of the number of broody chickens to the total number of certain genotype.

**Table 1.** Characteristics of the 8 chicken populations

| Population | Origin | Breeding purpose | Production performance |
|---|---|---|---|
| Red Jungle Fowls | Linshan County, Guangxi, China | Wild animal | Small size, seasonal reproduction and broodiness |
| Taihe Silkie | Taihe County, Jiangxi, China | Meat quality | Small size, a 70 to 80% incidence of broodiness |
| Xinghua chickens | Guangdong, China | Meat quality | Small size, a 70 to 80% incidence of broodiness |
| Ningdu Sanhuang chickens | Jiangxi, China | Meat quality | Small size, a 50 to 60% incidence of broodiness |
| Gushi chickens | Henan, China | Egg and meat production | Medium size, a 10 to 20% incidence of broodiness |
| Baier Huang chickens | Jiangxi, China | Egg production | Small size, a 15.4% incidence of broodiness |
| White Recessive Rock Broilers | Commercial broiler line imported from Kabir Co. Ltd., Italy | Egg and meat production | Large size, no broodiness in cage |
| Leghorn Layers | Commercial layer line derived from Italy | High egg production | Small size, early mature, no broodiness |

## Primers and Selection of Candidate Markers

According to the published mRNA sequences (Genbank accession number: XM_418492) of the chicken *VIPR-1* gene, and lately released chicken draft genome sequences (http://mgc.ucsc.edu/cgi-bin/hgBlat; accessed Mar. 2007), 11 pairs of primers were designed and synthesized for cloning (Table 2).

The criterion of selected markers was followed the criterion of Xu et al. (2005): (1) coding single nucleotide polymorphisms (**SNP**); (2) coding nonsynonymous SNP; (3) SNP at potential transcription factor binding sites; (4) SNP at a splicing site or near exons; (5) PCR-RFLP markers; (6) the average distance between 2 adjacent markers is 5 kb. Twenty candidate markers including 16 markers identified in the present study and 4 SNP [C+10593T in intron 2 (rs15863121), G+18805T in intron 2 (rs15863134), C+31332T in intron 3 (rs14127626), and C+42913T in intron 6 (rs15863161)] from dbSNP database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&DB=snp; accessed Jan. 2006) were selected to be genotyped. Eighteen pairs of primers were designed and synthesized for the above SNP genotyping (Table 2).

## PCR Conditions

The PCR was performed in a 25-μL mixture containing 50 ng of chicken genomic DNA, 1 × PCR buffer, 12.5 pmol of primers (P1 ~ E13-2), 100 μ*M* of each dNTP, 1.5 m*M* $MgCl_2$ and 1.0 U Taq DNA polymerase (Sangon Biological Engineering Technology Company, Shanghai, China). PCR was run in a gradient Mastercycler (Eppendorf Limited, Hamburg, Germany) with the following conditions: 3 min at 94°C, followed by 35 cycles of 30 s at 94°C, 45 s at annealing temperature (57.6 to 64.9°C; Table 2), 1 min at 72°C, and a final extension of 5 to 10 min at 72°C.

## SNP Identification

Amplified PCR products were purified and subcloned into the pMD18-T vector (TaKaRa Biotechnology Co., Ltd., Dalian, China). The DNA sequencing was performed using dye terminator chemistry on Applied Biosystem model 3700 sequencer by the dideoxy-mediated chain-termination method (Sanger et al., 1977). The cloned sequences were analyzed by using the software DNASTAR V 3.0 (Steve ShearDown, 1998–2001 version reserved by DNASTAR Inc., Madison, WI).

## Genotyping of the 20 Polymorphisms by PCR-RFLP

Genotypes of D+19820I were directly determined with 3% agarose gel electrophoresis for PCR product amplified by I2-4 primers. For the other polymorphisms, the PCR products were digested at 37°C overnight with *Csp6 I*, *FspB I*, *Mbo I*, *Tai I*, *Hpa II*, *Mbi I*, *Hha I*, *Msp I*, *Taq I*, *Xap I*, and *Mva I*, respectively. The digestion mixture contained 7-μL PCR products, 1 × digestion buffer, and 3.0 U of each enzyme. Their genotypes were then determined in TFM-40 Ultraviolet Transilluminator (UVP Company, Cambridge, UK) after 2.0% agarose gel electrophoresis for 30 min.

## Statistical Analyses

***Estimation of the Nucleotide Diversity and Identification of the Chicken VIPR-1 Gene Polymorphisms.*** We used DnaSP4.10 (Rozas et al., 2003) to analyze patterns of polymorphism and divergence and to perform tests of neutrality on the basis of the allele frequency spectrum. The tests included Tajima's D (Tajima, 1989) and Fu and Li's D and F (Fu and Li, 1993) test statistics. Positive selection or the presence of weakly deleterious mutations (as well as population growth) tends to give an excess of low frequency variants, resulting in negative test values. Balancing selection (or population contraction) may cause an excess of intermediate-frequency variants and positive test values.

Mutated sites were identified with MegAlign program of DNASTAR software (Steve Shear Down 1998–2001 version reserved by DNASTAR Inc., Madison, WI) by analyzing these sequences of the chicken *VIPR-1* gene.

**Table 2.** Details of primer pairs for the chicken *VIPR-1* gene

| Primer | Primers sequences (5′ → 3′) | Location[1] (nt)/sites | Length[2] (bp) | AT[3] (°C) | Restriction enzyme |
|---|---|---|---|---|---|
| P1 | F: CCGTGCCAACAGAACTGATGAC<br>R: ACGTTCAGCAGGCATCAGTG | 5′ flanking region:<br>−2,991 to −1,944 | 1,048 | 60 | / |
| P11 | F: CAGGGAAGTGTGTGTAGAGCAAAG<br>R: GGGGATCTCAGAGGGCTTTTAC | 5′ flanking region:<br>−2,036 to − 445 | 1,592 | 60 | / |
| P2 | F: GAGCAACAAAGTCATACAATCA<br>R: ATGGCACAGATGAAGCAGATG | −496 to +623 | 1,118 | 60 | / |
| P3 | F: CAGAAAGAGCATGCCTAGTTGTA<br>R: TGGAGATGTGGCACTGAGG | +19,601 to +20,162 | 562 | 59 | / |
| P4 | F: GAGCATGCCCAGTCTGTCTTG<br>R: CAGGAGTTCAAAGGGAGGAC | +31,522 to +31,845 | 324 | 61 | |
| P5 | F: CAGCAAGGAGGTCAGAACAAG | +35,317 to 35,366 | 1,249 | 58 | / |
| P6 | F: GCGATCTCAGAGTTACACACAG<br>R: TCTGCTGTGCTGGAGTATGTAG | +51,589 to +53,443 | 1,855 | 60 | / |
| P7 | F: GCAGAGGATTGGAACCAGAC<br>R: CCCAAGACAGACATTCAGTACA | +56,463 to +56,838 | 376 | 59 | / |
| P8 | F: CGGTTTACTGTGTGCTCCTAG<br>R: GGCCCATTTCTTGATTTCTG | +59,450 to +59,872 | 423 | 59 | / |
| P9 | F: GGCCCACATCACATATCGTAAC<br>R: GCTGCAAACTGTCTGGCTAG | +63,965 to +65,523 | 1,559 | 60 | / |
| P10 | F: CCCCTATGTACTCTCTCCTCTG<br>R: CTCCCCCGATCCCTAATG | +66,826 to +68,000 | 1,176 | 60 | / |
| 503 | F: CATGCGGGCTCTTGATCACTG<br>R: GATCAGGATTGGCAAAGCACTAC | C–1719T<br>A–1666G | 315 | 64.9 | *Csp6 I*<br>*FspB 1* |
| 502 | F: CCCCACTATGCCCACTGAC<br>R: AGGAACCACGAGCCAGATG | G–359T | 173 | 64.8 | *Mbo I* |
| 501 | F: TGAAAGCCCCCAGGATCT<br>R: GAGCAAAACAAAACCCAAATCA | A–284G | 365 | 58.2 | *Tai I* |
| E2 | F: TCGCCTGTCTCAATTAAATGTAC<br>R: GAGGGTCAGAGATCCAAGAAGT | A+457G (Ser18Gly) | 285 | 57.6 | *Hpa II* |
| 12-1 | F: AGAGGAACGCAGCCAGTG<br>R: CCCACCTAACATAAAAGCTCAAC | C+598T | 203 | 58.2 | *BsuR I* |
| I2-2 | F: CAGCATAAACTCCAGCCCATTC<br>R: TCCCTCCCAACTCAGACAATTC | C+10593T | 478 | 58.2 | *Tai I* |
| I2-3 | F: TGAGCTTTGCAACTGATATTAGAA<br>R: GCTGGTGTCATCATCAAGTCTG | G+18805T | 298 | 58.2 | *Hpa II* |
| I2-4 | F: GCCATCTTGCTCCCCCCTAC<br>R: GCAGCAAAGCCCTAAAAGCATT | I+19820D | 267 | 58.2 | *PCR* |
| E3 | F: CAGAAAGAGCATGCCTAGTTGTA<br>R: TGGAGATGTGGCACTGAGG | A+19928G<br>(Ala66Ala) | 562 | 59.0 | *Mbi I* |
| I3 | F: CACAAGCAAAGTGAAAGATGTA<br>R: AGCCCTGACTTGTGCAATTC | C+31332T | 432 | 59.8 | *Hha I* |
| I5 | F: CAGCAAGGAGGTCAGAACAAG<br>R: GGAGGAACCCAAATAGACATG | A+35542G<br>A+35573G | 359 | 58.2 | *Msp I*<br>*Csp6 I* |
| E6 | F: AATGGAAAGAGGTATGATGGAC<br>R: CAAAGCAATGTTCGGGTTCT | A+36264C<br>(Arg158Arg) | 412 | 57.6 | *Taq I* |
| I6-1 | F: GAGCCATTGCAGTCTTCATAAA<br>R: TTACCATCACAGAGGGGAAAG | C+37454T | 349 | 58.2 | *Xap I* |
| I6-2 | F: CCCCGTTAAACTCAGCAGAC<br>R: CCCAAAGTCCCACAAGGTAA | C+42913T | 434 | 58.2 | *Hha I* |
| I8 | F: CTCCTCAGGCAGACCATCATG<br>R: CTTGCACGTATCCTTGGGTAGC | C+53327T | 486 | 58.2 | *Taq I* |
| I10 | F: GGCCCACATCACATATCGTAAC<br>R: GACCCAACCACGAGCTCAAAG | C+64059T | 337 | 58.2 | *Csp6 I* |
| E13-1 | F: AGCGAAAGTGGCGGAGGTG<br>R: TGCCTCTTAGCTTGTCTGATCTTG | C+67117T | 222 | 63.3 | *Mva I* |
| E13-2 | F: GGGGTAAGGAAGGGAAGAGC<br>R: TCGGCTAACAAGGATGGTGAG | A+67608G | 363 | 61.9 | *Tai I* |

[1]Location referred to covered regions. The first nucleotide of the translation start codon was designated +1, with the next upstream nucleotide being –1.

[2]Length indicated PCR product length.

[3]AT referred to annealing temperature.

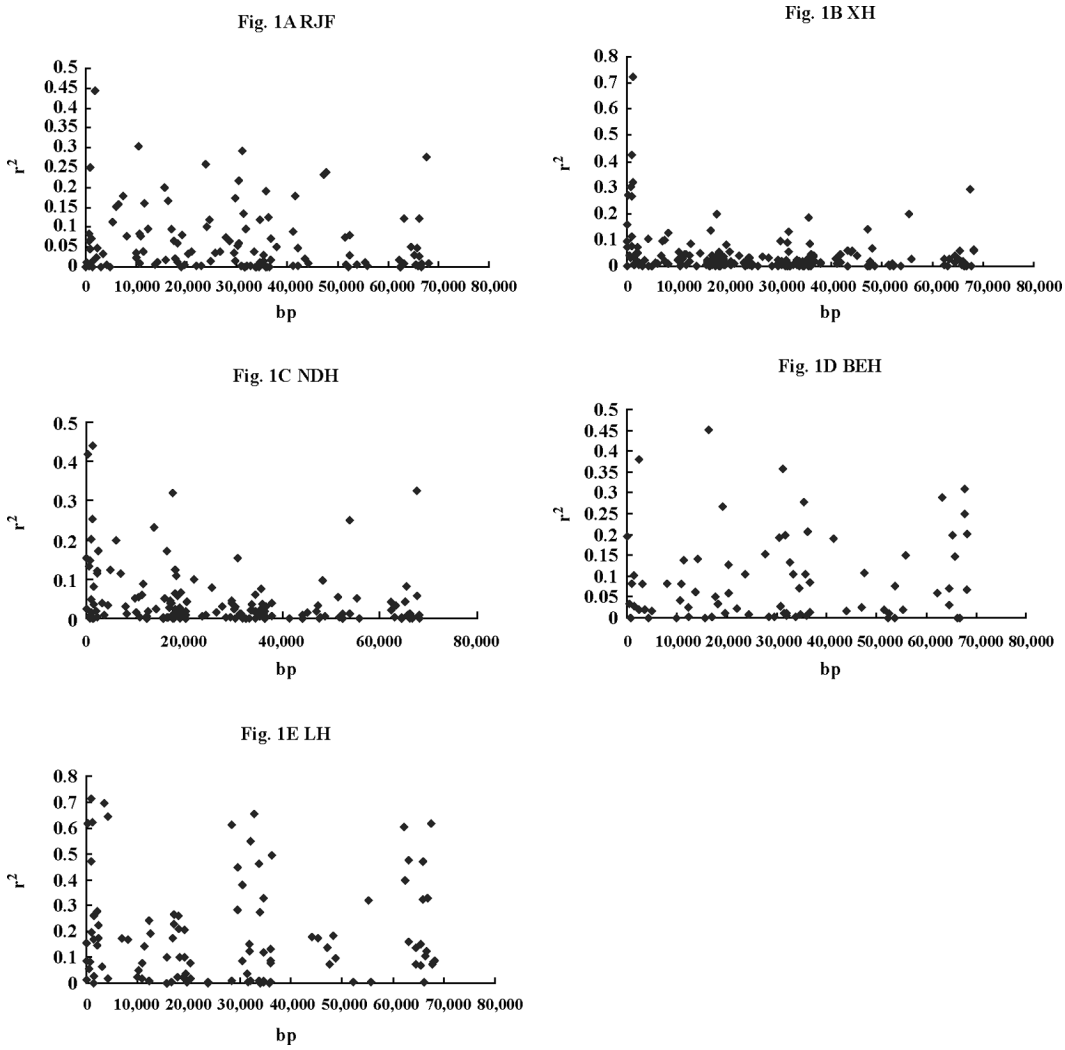


**Figure 1.** The correlation between $r^2$ and physical distance in the 5 chicken populations. RJF = Red Jungle Fowl; XH = Xinghua chickens; NDH = Ningdu Sanhuang chickens; BEH = Baier Huang chickens; LH = Likang Leghorn Layers.

***Prediction of the Transcription Factor Binding Sites in the 5′ Flanking Region of the Chicken VIPR-1 Gene.*** Prediction of potential binding sites induced by the 5′-flanking region polymorphisms was completed by 2 bioinformatic Web sites (http://www.gene-regulation.com/pub/programs/alibaba2 and http://motif.genome.jp). The sites identified by the 2 Web sites were the same. All parameters were set following the Web site standards.

***Linkage Disequilibrium and Haplotype Analyses.*** The Haploview version 3.32 software (http://www.broad.mit.edu/mpg/haploview/) was used to estimate the LD and infer haplotype block across the 20 polymorphisms. Genotypes of 364 random samples at the 20 polymorphisms were necessary for the application of Haploview. In each population, 2 loci were considered in LD ($r^2 \geq 0.33$), in strongly LD ($r^2 \geq 0.6$), in complete disequilibrium ($r^2 = 1$). The haplotype block was defined following the 4 gamete test (Wang et al., 2002).

***Allelic Frequency and Heterozygosity Calculation.*** Allelic frequencies and Nei's unbiased heterozygosity (**Hz**) based on the 20 polymorphisms in each population and in total were estimated using Microsatellite-Toolkit software (http://animalgenomics.ucd.ie/sdepark/ms-toolkit/). The Hardy-Weinberg equilibrium at each locus was assessed by simple $\chi^2$ test. Genotypes distribution between the 2 unrelated chicken populations was tested using Mantel-Haenszel ChiSquare (SAS 8.1 FREQ; SAS Institute Inc., Cary, NC).

***Marker-Trait Association Analyses.*** Association analyses of single polymorphism with DB were performed with SAS GLM procedure (SAS Institute Inc.), according to the following model:

$$Y_{ij} = \mu + G_i + H_j + E_{ij},$$

where $Y_{ij}$ is an observation on the traits, $\mu$ is the overall population mean, $G_i$ is the fixed effect associated with the ith genotype, $H_j$ is the fixed effect of hatch, and $E_{ij}$ is the random error. Multiple comparisons were analyzed with least squares means. The values were considered significant at $P \leq 0.05$ and presented as least squares means ± standard error means.

**Table 3.** Allelic frequencies of the 20 polymorphisms in the chicken *VIPR-1* gene in the 5 populations[1]

| Site | Allele | RJF (33) | XH (97) | NDH (96) | BEH (41) | LH (97) |
|---|---|---|---|---|---|---|
| C–1719T | T | 0.8636 | 0.2732 | 0.2083 | 0.1707 | 0.1392 |
| A–1666G | A | 0 | 0.4124* | 0.3698* | 0.5976* | 0.4330* |
| G–359T | G | 0.7121 | 0.6649 | 0.4323 | 0.4756 | 0.3763 |
| A–284G | A | 0.2121 | 0.1186 | 0.0625 | 0 | 0.4433* |
| A+457G | A | 0.8030 | 0.7474 | 0.7604 | 0.9634 | 0.4381 |
| C+598T | C | 0.4697 | 0.4742 | 0.6146 | 0.8537 | 0.3247 |
| C+10593T | C | 0.6212 | 0.5206 | 0.7500 | 0.8293 | 0.4381* |
| G+18805T | G | 0 | 0.4278 | 0.1979 | 0.0366 | 0.3557 |
| D+198201 | D | 0 | 0.1289 | 0.1667 | 0 | 0 |
| A+19928G | G | 0.2879 | 0.4948 | 0.2865 | 0.3902 | 0.3660 |
| C+31332T | C | 0.6212 | 0.3557 | 0.3021 | 0.4390 | 0.2938 |
| A+35542G | A | 0.7727 | 0.3041* | 0 | 0.8780* | 0.2371 |
| A+35573G | A | 0.9848 | 0.9021 | 0.9427 | 0.9634 | 0.7216 |
| A+36264C | A | 0.5303 | 0.2577 | 0.3229 | 0.2927 | 0.0052 |
| C+37454T | T | 0.1515 | 0.3144 | 0.3854 | 0.0122 | 0 |
| C+42913T | T | 0.1515 | 0.0773 | 0.0313 | 0.0122 | 0 |
| C+53327T | T | 0.2273 | 0.2320 | 0.5521 | 0.1098 | 0.0103 |
| C+64059T | T | 0.1818 | 0.0515* | 0.0625 | 0.2561 | 0.2371 |
| C+67117T | T | 0.3636 | 0.4588* | 0.4219* | 0.1951 | 0.4639* |
| A+67608G | G | 0.5758 | 0.2990 | 0.4479 | 0.5366 | 0.3093 |

[1]The first nucleotide of the translation start codon was designated +1, with the next upstream nucleotide being –1; RJF = Red Jungle Fowls; XH = Xinghua chickens; NDH = Ningdu Sanhuang chickens; BEH = Baier Huang chickens; LH = Likang Leghorn Layers. (n): sample sizes. Hardy-Weinberg deviation at the level of 0.05 was highlighted by asterisks (*).

Comparisons between groups for frequency of the broodiness vs. nonbroodiness hens were made by a $\chi^2$ test.

## RESULTS

### The Nucleotide Diversity of the Chicken VIPR-1 Gene

The average nucleotide diversity ($\pi$) calculated from the average number of pairwise difference was 0.00669 ± 0.00093 in RJF, and 0.00582 ± 0.00026 in domestic chickens. The $\pi$ value of the chicken *VIPR-1* gene in domestic chickens was lower 13.0% than that in RJF. Neutrality tests indicated that the $D_T$, $D_{FL}$, and $F_{FL}$, values of domestic chickens were negative, and those of RJF were only slightly positive and close to zero. The $D_T$, $D_{FL}$, and $F_{FL}$ values were not significantly different between RJF and domestic chickens (Supplemental Table 1).

### Polymorphisms of the Chicken VIPR-1 Gene

One hundred twenty-eight variation sites were found in the 11,136-bp region of the chicken *VIPR-1* gene, including 109 SNP and 19 other variations (Supplemental Tables 2 and 3). In the chicken *VIPR-1* gene, every 102 bp generated 1 SNP on average. Compared SNP density of each region, the 5′ flanking region had the highest variation rate, every 83 bp generated 1 SNP on average; and then the intron, every 93 bp generated 1 SNP on average. Three SNP in exon2, exon3, and exon6 were found in CDS of the *VIPR-1* gene; one in exon2 (A+457G) altered the translated mature protein (Ser18Gly).

### Prediction of Transcription Factor Binding Sites

Nine polymorphisms might make transcription factor binding sites disappear or change among 43 variation sites in the 5′ flanking region (Supplemental Table 4).

### LD Pattern and Haplotype Structure

The RJF, XH, NDH, and BEH exhibited distinct characteristic of decreasing $r^2$ value over physical distance. The effective extent of LD in the above 4 populations was 1,912 bp, 1,123 bp, 1,190 bp, and 31,344 bp, respectively, and the effective extent of LD in LH could not be generated (Figure 1). Haplotype analyses showed that the 27-kb region from exon 6 to exon 11 had some sites that were associated with broodiness (Figure 2).

### Allelic Frequencies and Hz

The allelic frequencies and Hz in the 5 populations were summarized in Tables 3 and 4. The $\chi^2$ testing for genotypes distribution between populations was summarized in Supplemental Table 5. The above data suggested that A–284G, A+457G, C+598T, D+19820I, C+37454T, C+42913T, and C+53327T might be associated with broodiness.

### Association of the VIPR-1 Gene Polymorphisms with Chicken Broodiness Traits

The larger variation was DB in the phenotypic performance in NDH population, in which the coefficients of variation were 185.11. Marker-trait association analyses


Fig. 2A Haplotype diversity of RJF

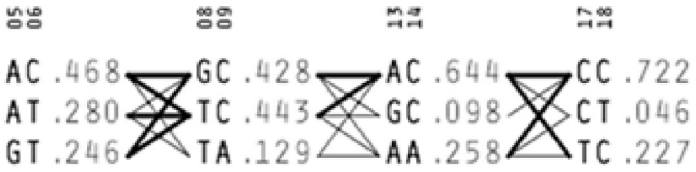


Fig. 2B Haplotype diversity of XH

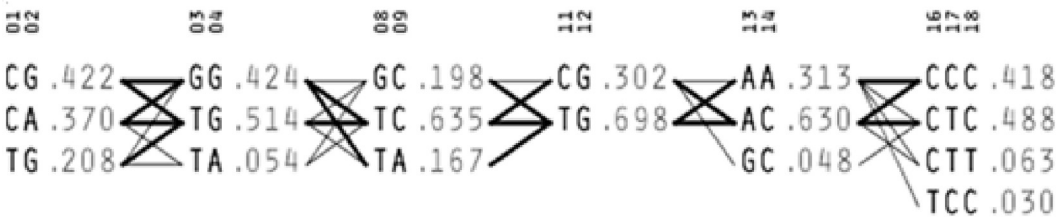


Fig. 2C Haplotype diversity of NDH

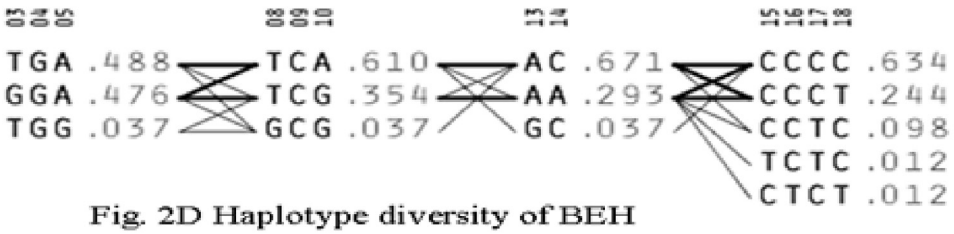


Fig. 2D Haplotype diversity of BEH

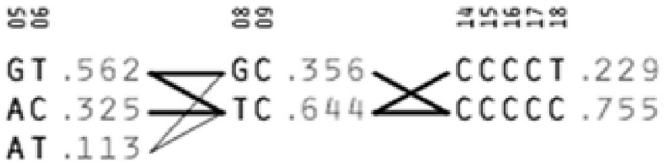


**Figure 2.** Haplotype diversity of the 5 chicken populations. 01: C-1719T; 02: A-1666G; 03: G-359T; 04: A-284G; 05: A+457G; 06: C+598T; 07: C+10593T; 08: G+18805T; 09: D+19820I; 10: A+19928G; 11: C+31332T; 12: A+35542G; 13: A+35573G; 14: A+36264C; 15: C+37454T; 16: C+42913T; 17: C+53327T; 18: C+64059T. RJF = Red Jungle Fowl; XH = Xinghua chickens; NDH = Ningdu Sanhuang chickens; BEH = Baier Huang chickens.

**Table 4.** Expected heterozygosities of the 20 polymorphisms in the chicken *VIPR-I* gene in the 5 populations[1]

| Site | RJF (33) | XH (97) | NDH (96) | BEH (41) | LH (97) |
|---|---|---|---|---|---|
| C–1719T | 0.2392 | 0.3992 | 0.3316 | 0.2867 | 0.2409 |
| A–1666G | 0 | 0.4872 | 0.4685 | 0.4869 | 0.4936 |
| G–359T | 0.4163 | 0.4479 | 0.4934 | 0.5050 | 0.4718 |
| A–284G | 0.3394 | 0.2101 | 0.1178 | 0 | 0.4961 |
| A+457G | 0.3212 | 0.3795 | 0.3663 | 0.0714 | 0.4949 |
| C+598T | 0.5038 | 0.5013 | 0.4762 | 0.2529 | 0.4408 |
| C+10593T | 0.4779 | 0.5017 | 0.3770 | 0.2867 | 0.4949 |
| G+18805T | 0 | 0.4921 | 0.3192 | 0.0714 | 0.4607 |
| D+198201 | 0 | 0.2257 | 0.2792 | 0 | 0 |
| A+19928G | 0.4163 | 0.5025 | 0.4109 | 0.4818 | 0.4665 |
| C+31332T | 0.4779 | 0.4607 | 0.4239 | 0.4986 | 0.4171 |
| A+35542G | 0.3566 | 0.4255 | 0 | 0.2168 | 0.3637 |
| A+35573G | 0.0303 | 0.1776 | 0.1086 | 0.0714 | 0.4038 |
| A+36264C | 0.5058 | 0.3856 | 0.4396 | 0.4192 | 0.0103 |
| C+37454T | 0.2611 | 0.4334 | 0.4762 | 0.0244 | 0 |
| C+42913T | 0.2611 | 0.1434 | 0.0609 | 0.0244 | 0 |
| C+53327T | 0.3566 | 0.3582 | 0.4972 | 0.1978 | 0.0205 |
| C+64059T | 0.3021 | 0.0983 | 0.1178 | 0.3857 | 0.3637 |
| C+67117T | 0.4699 | 0.4992 | 0.4903 | 0.3180 | 0.5000 |
| A+67608G | 0.4960 | 0.4213 | 0.4972 | 0.5035 | 0.4295 |
| Mean | 0.3117 ± 0.0697 | 0.3775 ± 0.0844 | 0.3376 ± 0.0755 | 0.2551 ± 0.0570 | 0.3284 ± 0.0734 |
| Range | 0 to 0.5058 | 0.0983 to 0.5025 | 0 to 0.4972 | 0 to 0.5050 | 0 to 0.5000 |

[1]The first nucleotide of the translation start codon was designated +1, with the next upstream nucleotide being –1. (n): sample sizes. RJF = Red Jungle Fowls; XH = Xinghua chickens; NDH = Ningdu Sanhuang chickens; BEH = Baier Huang chickens; LH = Likang Leghorn Layers.

showed that a significant association ($P < 0.05$) was found between C+598T in intron 2 and broody frequency (%; Table 5); a significant association ($P < 0.05$) was found between C+53327T and DB, allele C was positive for DB (Table 6). No significant association of the other sites with any chicken broodiness traits was found ($P > 0.05$; Table 7). A suggestive association ($0.05 < P < 0.1$) was found between D+19820I and DB, and allele D rather than I was dominant for chicken broodiness.

## DISCUSSION

The PRL appears to act centrally to initiate and maintain incubation behavior in birds. PRL secretion is controlled by VIP at the hypothalamus level and in part by VIP receptors at the pituitary level throughout the avian reproductive cycle (Chaiseha et al., 2004). In the present study, we demonstrated that the *VIPR-1* gene had endured stronger selection pressure during domestication and improvement under chicken breeding, and its DNA variations were associated with chicken broodiness. The *VIPR-1* gene can be acted as an MAS marker of reducing incidence of broodiness and improving egg production.

During domestication and improvement, humans exercised extremely strong selective pressure on ancestral gene pools to achieve desired phenotypic characteristics. These beneficial phenotypes were therefore fixed in the founder population of domesticated species in a short time (Innan and Kim, 2004). The domestication history of chickens is about 5,000 to 10,000 yr, and all kinds of domestic breeds had been developed during long domestication. Variations of chickens resulted from factors such as mutation, selection, migration, genetic drift, bottleneck, and effective population size (Fumihito et al., 1994; International Chicken Polymorphism Map Consortium, 2004). In this study, the average genetic diversity $\pi$ for the *VIPR-1* gene was 0.00669 in RJF and 0.00582 in domestic chickens. The domestic ones showed lower nucleotide diversity than the wild-

**Table 5.** Association of C+598T with incubation behavior in Ningdu Sanhuang chickens under cage conditions

| Trait | *P*-value | CC[1] (210) | CT[1] (333) | TT[1] (101) | a[2] ± SE | d[3] ± SE |
|---|---|---|---|---|---|---|
| DB[4] (d) | 0.8424 | 8.17 ± 0.83 | 7.56 ± 0.66 | 7.76 ± 1.17 | 1.226 ± 0.139 | –1.687 ± 0.006 |
| Number of nonbroody | — | 94 | 187 | 51 | — | — |
| Number of broody | — | 116 | 146 | 50 | — | — |
| Broodiness (%) | — | 0.5524 | 0.4384 | 0.4950 | — | — |
| $\chi^2$ value | <0.05 | 6.75* | — | — | — | — |

[1]Least squares means ± SE.

[2]Additive effect ± SE.

[3]Dominance ± SE. Number in brackets show the numbers of tested individuals of each genotype. $\chi^2_{0.05}$ (df = 2) = 5.99, $\chi^2_{0.01}$ (df = 2) = 9.21.

[4]DB = duration of broodiness; broodiness (%) was expressed as the percentage of the number of broody chickens to the total number of certain genotype.

*Indicated $P < 0.05$.

**Table 6.** Association of C+53327T with incubation behavior in Ningdu Sanhuang chickens under cage condition

| Trait | *P*-value | CC[1] (268) | CT[1] (273) | TT[1] (103) | a[2] ± SE | d[3] ± SE |
|---|---|---|---|---|---|---|
| DB[4] (d) | 0.0259* | 9.20 ± 0.71[a] | 6.72 ± 0.73[b] | 6.58 ± 1.16[b] | 2.449 ± 0.118 | −1.056 ± 0.006 |
| Number of nonbroody | — | 139 | 140 | 53 | — | — |
| Number of broody | — | 129 | 133 | 50 | — | — |
| Broodiness (%) | — | 0.4813 | 0.4872 | 0.4854 | — | — |
| $\chi^2$ value | >0.05 | 0.02 | — | — | — | — |

[a,b]Values within a row with no common superscript differ significantly ($P < 0.05$) or highly significantly ($P < 0.01$). Number in parentheses showed the numbers of tested individuals of each genotype.

[1]Least squares means ± SE.

[2]Additive effect ± SE.

[3]Dominance ± SE.

[4]DB = duration of broodiness; broodiness (%) was expressed as the percentage of the number of broody chickens to the total number of certain genotype.

*Indicated $P < 0.05$. $\chi^2_{0.05}$ (df = 2) = 5.99.

type population. No significant deviations from neutrality were detected in the D and *F*-test. Although the test statistic provided little statistical support for selection in the *VIPR-1* region, these statistics were known to have low power to detect selection and could be influenced by additional population genetic and demographic factors (Kreitman, 2000). As descriptive statistics, however, the negative values in domestic chickens relative to RJF were consistent with the expectation of a selective sweep. The $\pi$ value in RJF is inconsistent with a previous study, which showed that the $\pi$ value was 0.0065 at autosomal loci (Sundström et al., 2004), and that the genetic diversity in domestic chickens was 10.5% lower than that in this study, and was similar to that in RJF. This might be due to the fact that selection based on different aims could cause the fixation of benefit alleles.

Rich polymorphisms were found in the chicken *VIPR-1* gene. In the present study, the *VIPR-1* gene was scanned in the length of 11,136 bp, and 128 polymorphisms were found. Only 3 SNP were located in the coding region, but 64 located in the intron, which showed that the sequences of the coding region was conservative (Cargill et al., 1999; International Chicken Polymorphism Map Consortium, 2004). Mutation in transcriptional region and 5′ flanking region might cause the change of the expression at transcriptional level (Xu et al., 2005). Polymorphisms in the 5′ regulatory region of the *VIPR-1* gene were rich, and these sites might play an important role in regulating the *VIPR-1* gene transcription.

Not only the degree of LD was significantly different in different region of gene, but also different populations showed different patterns of pairwise LD (Stephens et al., 2001; Flint-Garcia et al., 2003; Palaisa et al., 2003; Jung et al., 2004; Nakamoto et al., 2006). Selection during domestication and improvement could influence the LD level of a gene (Saunders et al., 2002, 2005; Toomajian and Kreitman, 2002), and selection aiming at alleles of structural gene could increase the LD level in the target gene region significantly (Clark et al., 2004). Chicken displayed large phenotypic differences in broodiness. In Chinese native breeds, BEH chicken is known as a layer breed with lower incidence of broodiness by long-term selection to increase egg production during long domestication and improvement. The TS and XH chickens, selected for purification and rejuvenation to improve their productions and without selection for broodiness, are broiler breeds with 70 to 80% incidence of broodiness. The LH is an excellent layer breed without broodiness by systematic selection to increase egg production and to decrease broodiness during breeding. This study compared the effective extent of LD in BEH, which was selected against broodiness, with that of 3 other populations, which were never selected against broodiness; the former was 20 to 30 times the latter, and the effective extent of LD in LH, which was strongly selected against broodiness, could not be generated. In theory, LH had undergone long-term strong selection to minimize phenotypic expression of incubation behavior, and its diversity was the least. The extent of LD in LH should be bigger than that in native breeds. No effective extent of LD in LH was reasonable for the *VIPR-1* gene region. The results of average diversity also showed that the diversity in BEH was the least among the 5 populations, as much as lower 22.32% than that in LH. The *VIPR-1* gene in BEH might have endured stronger selection pressure during breeding for decreasing broodiness. The patterns of LD in this study were consistent with the patterns in maize (Tenaillon et al., 2001; Remington et al., 2001; Palaisa et al., 2003; Rafalski and Morgante, 2004). The haplotype block analyses in the 5 populations proved it too. When a genomic region was subjected to a recent selective sweep, such a sweep would have resulted in a reduced variability at this locus only (Schlötterer, 2003). In the 27-kb region from exon 6 to exon 11, with the selective pressure increasing against broodiness, the LD decayed more and more slowly, and the haplotype block was bigger and bigger. In the 5 populations, no haplotype block was developed as the LD decayed rapidly in RJF, which was not selected against broodiness, and the haplotype block was 27 kb in LH, which was selected strongly against broodiness, the same in BEH (Figure 2). Such result was consistent with the Remington et al. (2001). We presumed that hitchhiking happened in the 27-kb region.

Genotype data of 364 random samples at the 20 polymorphisms provided information to investigate the genetic characterization of the chicken *VIPR-1* gene. In this study, we found that among genotype distribution of the 20 poly-

**Table 7.** The probability of association (*P*-value) of the 12 polymorphic markers in the *VIPR-1* gene with chicken broodiness traits in Ningdu Sanhuang chickens under cage condition[1]

| Polymorphism | Duration of broodiness (d) | Broodiness (%) |
|---|---|---|
| C–1719T | NS | NS |
| A–284G | NS | NS |
| A+457G | NS | NS |
| C+598T | NS | $0.01 < P < 0.05$ |
| G+17606T | NS | NS |
| I+18621D | 0.0675 | NS |
| A+18729G | NS | NS |
| A+5065C | NS | NS |
| C+36255T | NS | NS |
| C+41714T | NS | NS |
| C+52128T | 0.0259 | NS |
| A+66409G | NS | NS |

[1]Broodiness (%) was expressed as the percentage of the number of broody chickens to the total number of certain genotype. NS = not significant ($P > 0.05$).

morphisms in LH, only that of A+19928G was less different than that in others (Supplemental Table 5), which indirectly proved that LH was highly different from the Chinese breeds. Considering their genetic differences, we thought that BEH should be a better control population than LH to find markers associated with broodiness based on distribution and variation tendency of allelic and genotypic frequency, and genetic diversity in this study. Based on distribution and variation tendency of allelic and genotypic frequencies, there was significant difference for allelic frequencies and genotypic frequencies of the 6 polymorphisms, G–359T, A–284G, A+457G, C+598T, C+10593T, and C+37454T, among the populations with higher broodiness such as RJF, XH, and NDH and the populations with lower broodiness such as BEH. If the *VIPR-1* gene endured stronger selection pressure during breeding against broodiness, these polymorphisms associated with this behavior should have a lower Hz. Based on the Hz values, 7 polymorphisms, A–284G, A+457G, C+598T, D+19820I, C+37454T, C+42913T, and C+53327T, might be associated with broodiness. Although distribution and variation tendency of allelic and genotypic frequencies of C+42913T and C+53327T were not significantly different among 5 populations, it was similar between BEH and LH that were selected against broodiness, for which we assumed the 2 SNP were associated with broodiness. Considered together, 7 interesting polymorphisms, A–284G, A+457G, C+598T, D+19820I, C+37454T, C+42913T, and C+53327T, might be associated with broodiness.

The objectives of the current study were to identify polymorphisms for incubation behavior within a biological candidate gene and to determine if these markers are associated with broodiness traits. Taken together, the above results suggest that some DNA variation in the *VIPR-1* gene might be candidate markers associated with chicken broodiness traits. The variations are A–284G, A+457G, C+598T, D+19820I, C+37454T, C+42913T, and C+53327T, respectively, in which 6 were in the 5′ flanking region, in the intron, or both. Recently, functional elements were found in gene introns and that elements could regulate gene expression (Haddrill et al., 2005; Wang and Shashikant, 2007). The association results showed that C+598T in intron 2 and C+53327T in intron 8 were associated with chicken broodiness.

Taken together, our findings provide valuable knowledge of the *VIPR-1* gene endured stronger selection pressure during chicken domestication and improvement in part. The DNA variation in the *VIPR-1* gene was associated with chicken broodiness. In conclusion, a total of 109 SNP and 19 other variations were identified in the chicken *VIPR-1* gene, and C+598T and C+53327T were significantly associated with broodiness.

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## REFERENCES

Cargill, M., D. Altshuler, J. Ireland, P. Sklar, K. Ardlie, N. Patil, N. Shaw, C. R. Lane, E. P. Lim, N. Kalyanaraman, J. Nemesh, L. Ziaugra, L. Friedland, A. Rolfe, J. Warrington, R. Lipshutz, G. Q. Daley, and E. S. Lander. 1999. Characterization of single-nucleotide polymorphisms in coding regions of human genes. Nat. Genet. 22:231–238.

Chaiseha, Y., O. M. Youngren, and M. E. El Halawani. 2004. Expression of vasoactive intestinal peptide receptor messenger RNA in the hypothalamus and pituitary throughout the turkey reproductive cycle. Biol. Reprod. 70:593–599.

Clark, R. M., E. Linton, J. Messing, and J. F. Doebley. 2004. Pattern of diversity in the genomic region near the maize domestication gene tb1. Proc. Natl. Acad. Sci. USA 101:700–707.

El Halawani, M. E., and I. Rozenboim. 1993. The ontogeny and control of incubation behavior in turkeys. Poult. Sci. 72:906–911.

El Halawani, M. E., J. L. Silsby, and L. J. Mauro. 1990. Vasoactive intestinal peptide is a hypothalamic prolactin releasing neuropeptide in the turkey (*Meleagris gallopavo*). Gen. Comp. Endocrinol. 78:66–73.

El Halawani, M. E., J. L. Silsby, I. Rozenboim, and G. R. Pitts. 1995. Increased egg production by active immunization against vasoactive intestinal peptide in the turkey (*Meleagris gallopavo*). Biol. Reprod. 52:179–183.

El Halawani, M. E., S. E. Whiting, J. L. Silsby, G. R. Pitts, and Y. Chaiseha. 2000. Active immunization with vasoactive intestinal peptide in turkey hens. Poult. Sci. 79:349–354.

El Halawani, M. E., O. M. Youngren, and G. R. Pitts. 1997. Vasoactive intestinal peptide as the avian prolactin-releasing factor. Pages 403–416 in Perspectives in Avian Endocrinology, S. Harvey, and R. J. Etches, ed. Journal of Endocrinology Ltd., Bristol, UK.

Flint-Garcia, S. A., J. M. Thornsberry, and E. S. Buckler. 2003. Structure of linkage disequilibrium in plants. Annu. Rev. Plant Biol. 54:357–374.

Fu, Y. X., and W. H. Li. 1993. Statistical tests of neutrality of mutations. Genetics 133:693–709.

Fumihito, A., T. Mivake, S. Sumi, M. Takadk, S. Ohno, and N. Kondo. 1994. 1994. One subspecies of the red junglefowl (*Gallus gallus gallus*) suffices as the matriarchic ancestor of all domestic breeds. Proc. Natl. Acad. Sci. USA 91:12505–12509.

Gaudin, P., J. J. Maoret, A. Couvineau, C. Rouver-Fessard, and M. Laburthe. 1998. Constitutive activation of the human vasoactive intestinal peptide 1 receptor, a member of the new

class II family of G protein-coupled receptors. J. Biol. Chem. 273:4990–4996.
Haddrill, P. R., B. Charlesworth, D. L. Halligan, and P. Andolfatto. 2005. Patterns of intron sequence evolution in *Drosophila* are dependent upon length and GC content. Genome Biol. 6:R67.
Innan, H., and Y. Kim. 2004. Pattern of polymorphism after strong artificial selection in a domestication event. Proc. Natl. Acad. Sci. USA 101:10667–10672.
International Chicken Polymorphism Map Consortium. 2004. A genetic variation map for chicken with 2.8 million single-nucleotide polymorphisms. Nature 432:717–722.
Jiang, R. S., G. Y. Xu, X. Q. Zhang, and N. Yang. 2005. Association of polymorphisms for prolactin and prolactin receptor genes with broody traits in chickens. Poult. Sci. 84:839–845.
Jung, M., A. Ching, D. Bhatttramakki, M. Dolan, S. Tingey, M. Morgante, and A. Rafalski. 2004. Linkage disequilibrium and sequence diversity in a 500-kbp region around the adh1 locus in elite maize germplasm. Theor. Appl. Genet. 109:681–689.
Kansaku, N., K. Shimada, T. Ohkubo, N. Saito, T. Suzuki, Y. Matsuda, and D. Zadworny. 2001. Molecular cloning of chicken vasoactive intestinal polypeptide receptor complementary DNA, tissue distribution and chromosomal localization. Biol. Reprod. 64:1575–1581.
Kreitman, M. 2000. Methods to detect selection in populations with applications to the human. Annu. Rev. Genomics Hum. Genet. 1:539–559.
Liang, Y., J. X. Cui, G. F. Yang, F. C. C. Leung, and X. Q. Zhang. 2006. Polymorphisms of 5′ flanking region of chicken prolactin gene. Domest. Anim. Endocrinol. 30:1–16.
Nakamoto, K., S. Wang, R. D. Jenison, G. L. Guo, C. D. Klaassen, Y. J. Wan, and X. B. Zhong. 2006. Linkage disequilibrium blocks, haplotype structure, and htSNPs of human CYP7A1 gene. BMC Genet. 7:1–12.
Palaisa, K. A., M. Morgante, M. Williams, and A. Rafalski. 2003. Contrasting effects of selection on sequence diversity and linkage disequilibrium at two phytoene synthase loci. Plant Cell 15:1795–1806.
Rafalski, A., and M. Morgante. 2004. Corn and humans: Recombination and linkage disequilibrium in two genomes of similar size. Trends Genet. 20:103–111.
Remington, D. L., J. M. Thornsberry, Y. Matsuoka, L. M. Wilson, S. R. Whitt, J. Doebley, S. Kresoyich, M. M. Goodman and E. S. Buckler 4th. 2001. Structure of linkage disequilibrium and phenotypic associations in the maize genome. Proc. Natl. Acad. Sci. USA. 98:11479–11484.
Romanov, M. N., R. T. Talbot, P. W. Wilson, and P. J. Sharp. 2002. Genetic control of incubation behavior in the domestic hen. Poult. Sci. 81:928–931.
Rozas, J., J. C. Sáchez-Delbarrio, X. Messeguer, and R. Rozas. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496–2497.
Rozenboim, I., and M. E. El Halawani. 1993. Chracterization of vasoactive intestinal peptide pituitary membrane receptors in turkey hens during different stages of reproduction. Biol. Reprod. 48:1129–1134.
Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74:5463–5467.
Saunders, M. A., M. F. Hammer, and M. W. Nachman. 2002. Nucleotide variability at G6PD and the signature of malarial selection in humans. Genetics 162:1849–1861.
Saunders, M. A., M. Slatkin, C. Gaener, M. F. Hammer, and M. W. Nachman. 2005. The extent of linkage disequilibrium caused by selection on G6PD in humans. Genetics 171:1219–1229.
Schlötterer, C. 2003. Hitchhiking mapping-functional genomics from the population genetics perspective. Trends Genet. 19:32–38.
Sharp, P. J. 2004. Genes for persistency of egg laying: White Leghorns and broodiness. Roslin Institute Edinburgh Annual Report: 38–42.
Sharp, P. J., M. C. MacNamee, R. T. Talbot, R. J. Sterling, and T. R. Hall. 1984. Aspects of the neuroendocrine control of ovulation and broodiness in the domestic hen. J. Exp. Zool. 232:475–483.
Sharp, P. J., R. J. Sterling, R. T. Talbot, and N. S. Huskisson. 1989. The role of hypothalamic vasoactive intestinal polypeptide in the maintenance of prolactin secretion in incubating bantam hens: Observations using passive immunization, radioimmunoassay and immunohistochemistry. J. Endocrinol. 122:5–13.
Stephens, J. C., J. A. Schneider, D. A. Tanquay, J. Choi, T. Acharya, S. E. Stanley, R. Jiang, C. J. Messer, A. Chew, J. H. Han, J. Duan, J. L. Carr, M. S. Lee, B. Koshy, A. M. Kumar, G. Zhang, W. R. Newell, A. Windemuth, C. Xu, T. S. Kalbfleisch, S. L. Shaner, K. Arnold, V. Schulz, C. M. Drysdale, K. Nandabalan, R. S. Judson, G. Ruano, and G. F. Vovis. 2001. Haplotype variation and linkage disequilibrium in 313 human genes. Science 293:489–492.
Sundström, H., M. T. Webster, and H. Ellegren. 2004. Reduced variation on the chicken Z chromosome. Genetics 167:377–385.
Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595.
Tenaillon, M. I., M. C. Sawkins, A. D. Long, R. L. Gaut, J. F. Doebley, and B. S. Gaut. 2001. Patterns of DNA sequence polymorphism along chromosome 1 of maize (*Zea mays* ssp. *mays* L.). Proc. Natl. Acad. Sci. USA 98:9161–9166.
Toomajian, C., and M. Kreitman. 2002. Sequence variation and haplotype structure at the human HFE locus. Genetics 161:1609–1623.
Wang, N., J. M. Akey, K. Zhang, R. Chakraborty, and L. Jin. 2002. Distribution of recombination crossovers and the origin of haplotype blocks: The interplay of population history, recombination, and mutation. Am. J. Hum. Genet. 71:1227–1234.
Wang, W. C., and C. S. Shashikant. 2007. Evidence for positive and negative regulation of the mouse Cdx2 gene. J. Exp. Zool. B. Mol. Dev. Evol. 308:308–321.
Xu, H., S. G. Gregory, E. R. Hauser, J. E. Stenger, M. A. Pericak-Vance, J. M. Vance, S. Züchner, and M. A. Hauser. 2005. SNPselector: A web tool for selecting SNPs for genetic association studies. Bioinformatics 21:4181–4186.
Yamasaki, M., M. I. Tenaillon, I. V. Bi, S. G. Schroeder, H. Sanchez-Villeda, J. F. Doebley, B. S. Gaut, and M. D. McMullen. 2005. A large-scale screen for artificial selection in maize identifies candidate agronomic loci for domestication and crop improvement. Plant Cell 17:2859–2872.
You, S., C. C. Hsu, H. Kim, Y. Kho, Y. J. Choi, M. E. El Halawani, J. Farris, and D. N. Foster. 2001. Molecular cloning and expression analysis of the turkey vasoactive intestinal peptide receptor. Gen. Comp. Endocrinol. 124:53–65.