The Genetics and Evolution of Eye Color in Domestic Pigeons (*Columba livia*)

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Abstract

The avian eye color, generally referred to the color of the bird's iris, results from both pigments and structural coloration. Avian iris colors exhibit striking interspecies variations that may reflect unique evolutionary history and ecological adaption in different taxa. With a wide range of intraspecific iris color polymorphism, the domestic pigeon (*Columba livia*) thus serves as an excellent model for elucidating the largely unknown genetic mechanism underlying the evolution of eye color in birds. Here we tackled the genetics of the pearl (white) iris color, a recessive trait characterized by an absence of yellow pigment (pteridine) in pigeons' eyes. Using a genome wide association study (GWAS) on 49 wild-type and 43 pearl-eyed pigeons, we identified a 9 kb genomic region associated with the pearl iris, within which only one gene *SLC2A11B* was located. A nonsense mutation W49X in SLC2A11B was further identified as the causal variant leading to the pearl iris color. *SLC2A11B* encodes a facilitative glucose transporter and is known to be involved in xanthophore and leucophore differentiation in fishes. Transcriptome analysis suggested that loss-of-function of SLC2A11B affected the differentiation of the xanthophore-like pigment cell in pigeon irises by downregulating *CSF1R*, and turned off the biosynthesis of pteridine by inhibiting GCH1. Coalescence and evolutionary analyses of *SLC2A11B* indicated the pearl iris mutation was originated about 5,400 years ago in congruence with the time of pigeon domestication, and signal of positive selection was detected likely associated with artificial selective breeding. A fixed frameshift mutation in *SLC2A11B* was also found in cormorant species corresponding to their signature blue eyes. This study provided new insight into the mechanism of avian iris color variations and shed light on the evolution of pigment cells in vertebrates.

Key words: domestic pigeon, iris color, genome, domestication, evolution

Introduction

Integumentary pigmentation of vertebrates plays an essential role in camouflage, sexual selection, communication and thermoregulation (Hoekstra, 2006; Hofreiter and Schoneberg, 2010; Hubbard *et al.* 2010). The dynamic color change and diverse static integumentary pigmentation of poikilothermic vertebrates is mostly attributed to the neural crest-derived dermal chromatophores, which are generally divided into three main categories: xanthophore/erythrophore (yellow to red), iridophore/leucophore (irisescent color or white) and melanophore (black) (Grether *et al.,* 2004; Kelsh, 2004; Olsson *et al.,* 2013).

The eye color of a bird, usually referred to the color of the bird's iris, derived from both pigmentation and structural coloration such as the diffraction of light. In birds, with the outer insulating feather coverage that masks the skin pigmentation, the dermal chromatophores seem to lose selective advantage and may be subject to evolutionary demise (Oliphant *et al.,* 1992). However, the avian iris maintains the potential for complete development of all types of pigment cells that are comparable to the chromatophores in vertebrates, probably due to its external, exposed location where chromatophores are under constant selective pressure, thus remaining as a pigment cell refugium during avian evolution (Oliphant *et al.,* 1992).

The pigment cells are located in the stroma of the iris, the anterior layer of loose vascular connective tissue. A bird's eye color varies by the presence or absence of pigment cells in the iris and the pigment contents, and exhibit striking interspecies variations, ranging from black and dark brown, to brilliant colors covering nearly the full spectrum of rainbow (Bond, 1920; Oliphant, 1987a; Oliphant *et al.,* 1992). Although the adaptive advantage underlying the iris color variation in birds remains elusive, recent studies had shed light on the possible coevolution of iris color and behavior or activity rhythm in avian species (Craig and Hulley, 2004; Davidson *et al.,* 2014; Davidson *et al.,* 2017; Passarotto *et al.,* 2018). The iris color variation between species may reflect its unique history of evolution and adaption and thus provides a unique angle for understanding the avian radiation, as well as the evolution of pigmentation in vertebrates. However, the evolutionary drive shaping avian eye color remains largely unknown, as intraspecific color variation is rare and only common in domestic animals.

The domestic pigeon (*Columba livia*) was derived from its conspecific wild ancestor the rock pigeon, and the origin of domestication is believed to occur around 5,000 years ago in the Mediterranean region (Darwin, 1868; Price, 2002; Driscoll *et al.,* 2009). Since the initial domestication, pigeons have gone through intense selective breeding to have developed a tremendous array of phenotypic variations within the species (Darwin, 1868; Price, 2002; Domyan and Shapiro, 2017). Recent studies have revealed the molecular basis of some intraspecific variations in domestic pigeons such as plumage pigmentation, feather ornament, epidermal appendage, and navigation behavior, and highlighted the genes that might play important roles in avian evolution in these aspects (Shapiro *et al.,* 2013; Domyan *et al.,* 2014; Domyan *et al.,* 2016; Vickrey *et al.,* 2015; Vickrey *et al.,* 2018; Gazda *et al.,* 2018; Boer *et al.* 2019; Shao *et al.,* 2020; Bruders *et al.,* 2020).

The domestic pigeon exhibits three major types of iris color: the yellow to orange "gravel" eye (wild type, Fig. 1A), the white "pearl" eye (Fig. 1B), and the black "bull" eye that is most commonly observed in other avian species (Bond, 1920; Oliphant, 1987a; Oliphant *et al.,* 1992). The gravel and pearl irises in pigeons contain bright pigment cells with birefringent crystals in the anterior stroma tissue, while the bull eye results from the complete absence of stromal pigment cells (Oliphant, 1987b). Crystalline guanine was identified in both gravel and pearl irises as the major pigment, and at least two yellow fluorescing pteridine materials were identified in the gravel iris leading to the yellow color tone (Oliphant, 1987b). Therefore, the stromal pigment cell in the gravel iris was also referred as "reflecting xanthophore" (Oliphant, 1987b). The irises of all newly hatched pigeons were bull-eyed dark, while those with gravel or pearl eyes gradually grew into brighter color after two to three months. Breeding records and empirical studies have indicated the pearl eye in domestic pigeons followed an autosomal recessive inheritance mode, denoted as the *Tr* locus, and the bull eye was associated with white feather (Hollander and Owen, 1939). Similar stromal pigment cells and (or) developmental color changes were found in the irises of other avian species, suggesting the domestic pigeon is an ideal model to illuminate the evolution of avian iris color variations.

In this study, we investigated the genetic basis and evolution history of the bright irises (gravel and pearl eyes) in domestic pigeons. We identified the genetic mutation responsible for the iris color change from gravel to pearl, and found the pearl iris trait likely originated about 5,400 years ago as a domesticated trait subject to intense artificial selection. In addition, a fixed loss-of-function variant in the same gene was

detected in cormorant species, consistent with their signature blue iris due to the absence of pteridine pigment. This pigmentation gene was under strong selection in avian species suggesting its critical role in avian iris color evolution.

Results

Tr locus is mapped to a 9 Kb genomic region

A case-control genome-wide associate study (GWAS) based on whole genome resequencing was performed on domestic pigeons (racing homers) to identify the genomic region responsible for the bright iris phenotype. Whole genome sequencing data were retrieved from 49 gravel-eyed and 43 pearl-eyed individuals for an approximate 10× genome coverage each (Table S1). Of the 28,628,324 SNPs initially identified, 2,490,056 passed the genotype filtering and quality control and were used in subsequent analysis (Fig. S1). Significant association signals (p<2.01E-8, Bonferroni correction) were observed in 160 consecutive SNPs from the pigeon genome (Cliv_2.1) scaffold AKCR02000030.1 (hereafter referred as scaffold 30), which spanned a single 341 Kb region associated with the pearl-eyed phenotype (minimum p=1.07E-16) (Fig. 2A, S2). No SNP from other scaffold showed significant signal of association with the iris phenotypes (Fig. 2A, S2). In order to fine-map the genomic region for the *Tr* locus, haplotypes of the mapped region on scaffold 30 were built for each individual based on the whole genome data. While the wild-typed individuals exhibited multiple haplotypes at this region, a single 9 Kb haplotype (scaffold 30: 1894006-1903342) was shared by all 43 pearl-eyed pigeons, confirming it as the strong candidate region for the *Tr* locus (Fig. 2B).

A premature stop codon of *SLC2A11B* caused the pearl eye phenotype

The mapped 9 Kb interval of *Tr* locus contained only one protein coding gene according to the gene annotation of the reference genome (Cliv_2.1): *A306_00005299* (Fig. 2B). *A306_0000529* was annotated as solute carrier family 2 member 11-like, and was further identified as *SLC2A11B* (solute carrier family 2, facilitated glucose transporter, member 11b) based on the gene homology and synteny in vertebrates (avian, reptile and fish). *SLC2A11B* is known to play an essential role in the differentiation of leucophores and xanthophores in medaka fish (Kimura *et al.,* 2014) and hence a strong candidate gene for the pearl eye color in domestic pigeons. In order to identify the causative mutation, Screening of variations in the pigeon *SLC2A11B* coding region identified a G-to-A transition in the exon 3 that introduced a

premature stop codon (W49X) truncating the downstream 448 amino acids of SLC2A11B (Fig. 3, S3). Loss-of-function in *SLC2A11B* in medaka fish prevented the development of xanthophores and abolished the yellow pigment in leucophores at embryonic/larval stages (Kimura *et al.,* 2014). *SLC2A11B*-knockout zebrafish also exhibited reduced yellow pigment due to the defect of the xanthophore differentiation (https://zmp.buschlab.org/gene/ENSDARG00000093395). The iris pigmentation change in pearl-eyed pigeons thus resembles that of *SLC2A11B* loss-of-function fish, strongly supporting W49X of *SLC2A11B* as the causative mutation responsible for the pearl eye phenotype. This non-sense mutation was further validated in 146 gravel-eyed and 146 pearl-eyed individuals. All gravel-eyed pigeons carried at least one wild-type allele, while 141 out of the 146 pearl-eyed individuals were homozygous for the mutant allele consistent with its recessive mode of inheritance. However, five pearl-eyed pigeons were heterozygous for the SLC2A11B W49X variant (Table 1) and there was no other mutation found from the exons of *SLC2A11B*.

SLC2A11B W49X affected the stromal pigment cell differentiation in pigeons

The expression pattern of *SLC2A11B* was profiled in multiple tissues from nine wild-type domestic pigeons. Notably, the expression of *SLC2A11B* was high in iris tissue, but extremely low in skin and feather bud (Fig. 4A), indicating that the role of *SLC2A11B* in pigmentation was restricted to the iris in pigeons. In addition, high expression of *SLC2A11B* was also observed in brain, retina, and muscle (Fig. 4A), suggesting a pleiotropic effect of *SLC2A11B*.

In order to gain insight into the mechanism of the iris color change, we performed transcriptome analysis of iris tissues from four gravel and five pearl pigeons (Table S3). In total, 337 differentially expressed genes (DEGs) were identified ((FDR<0.05), Fig. 4B, S4, S5 Table S4). *SLC2A11B* was slightly downregulated by 1.2 fold in the pearl iris (Fig. 4B), probably caused by the nonsense-mutation-mediated RNA decay. Among these DEGs, we examined the genes involved in pteridine biosynthesize or xanthophore/leucophore differentiation. We identified that *GTP cyclohydrolase 1* (*GCH1)*, the gene encoding the rate-limiting enzyme responsible for pteridine biosynthesize, was significantly downregulated (3.6 fold) in the iris tissues of pearl individuals (Fig. 4B), corresponding to the absence of pteridine in irises of pearl individuals. We also found *colony stimulating factor 1 receptor* (*CSF1R*) was significantly downregulated (1.8 fold) in pearl irises (Fig. 4B). *CSF1R* encodes a receptor tyrosine kinase required for xanthophore differentiation (Kelsh *et al.,* 2010;

Patterson *et al.,* 2014; Singh and Nusslein-Volhard, 2015). All these three DEGs were confirmed by quantitative RT-PCR (Fig. 4C). These observations suggested that the loss-of-function of *SLC2A11B* may affect the differentiation of the xanthophore-like stromal pigment cells through downregulating *CSF1R*, resulting in the turn-off of the machinery of pteridine biosynthesis.

The pearl eye was a domesticated trait under artificial selection in pigeons

To understand the evolution and origin of pearl eye in pigeon population, the genome data of 36 fancy pigeons from 36 representative breeds (Shapiro *et al.,* 2013) was surveyed for the *SLC2A11B* mutation. The pearl-eyed variant *SLC2A11B* W49X was present in 19 of the 36 pigeon breeds with a 0.42 frequency ratio, suggesting a widespread prevalence and probably a relatively ancient origin of the pearl eye mutation in domestic pigeons (Table S2).

A neighbor-joining phylogenetic tree was reconstructed for 140 domestic pigeons (36 fancy pigeons, 2 feral pigeons, and 102 racing homers) and one *Columba rupestris*, based upon haplotypes across the full-length *SLC2A11B* gene region inferred from genome sequencing data (Fig. 5A). All haplotypes containing W49X mutation formed a single clade nested within the wild-type clade (Fig. 5A), suggesting a derived state of the *SLC2A11B* variants originating from the wild type via one single mutation event. In addition, mutant haplotypes carried by various breed groups or from different geographic affinities clustered together in the phylogenetic tree (Fig. 5A), suggesting that the mutation was ancient and occurred prior to the establishment of modern pigeon breeds. We further estimated the time to the most recent common ancestor (TMRCA) for all *SLC2A11B* haplotypes bearing the mutation at about 5,400 years ago (95% CI:4,700-5800 years ago) (Fig. 5B), a time period compatible with the time of pigeon domestication estimated at more than 5,000 years ago.

Tests of signal of selection in pigeons for the pearl eye trait were performed based on 140 pigeon genome data. We applied integrated haplotype score (iHS) analysis on the Scaffold 30 where *Tr* locus located, and identified continuous

selective signals at the *Tr* locus and its adjacent region (Scaffold 30: 1.4-2.0 Mb) (Fig. 6A). Nucleotide diversity(π), Tajima's D, extended haplotype homozygosity (EHH) were further calculated on the haplotypes spanning the potentially region under selection. Decreased nucleotide diversity was observed in the mutant haplotypes (the one with W49X mutation) at *Tr* locus where Tajima's D of the same haplotypes was also negative (Fig. 6C, 6D), consistent with a selective sweep scenario. The mutant haplotypes exhibited slower homozygosity decay than the wild-type ones by the EHH analysis (Fig. 6B), in support of the presence of selection for the mutant allele. These compelling evidences jointly illustrated that the prevalence of pearl iris trait in pigeons was likely a result of artificial selection after pigeon domestication.

SLC2A11B determines iris color of avian species

SLC2A11B plays an important role for the differentiation of xanthophore-like pigment cell in avian iris, thus the defect of *SLC2A11B* might be responsible for the iris color of some species that lack of pteridine pigment in their eyes. We extracted the coding sequences of *SLC2A11B* from published genome data of 76 avian species (Jarvis *et al.,* 2014), and screened variations that might affect the function of SLC2A11B protein. We found that a reading frameshift mutation T484Tfs2 was fixed in all five species of the cormorant lineage (*Nannopterum auratus, N brasilianus, N harrisi, Urile pelagicus, Phalacrocorax carbo*) (Fig. S7). The T484Tfs2 truncated the 12 amino acids at the C-terminus of SLC2A11B, most of which were evolutionary conserved, therefore, might impair the function of SLC2A11B. All the irises from the five cormorant species were signature blue showing no hint of yellow (pteridine) pigment. Neither pteridine nor purine was detected in the iris of *Nannopterum auratus*, whose blue color was presumed as a structural color generated by the unusual membrane-bound granules with electron-dense rods of the melanocyte-like cells (Oliphant *et al.,* 1992). It is likely that the T484Tfs2 impaired the function of SLC2A11B, causing an absence of mature xanthophore-like stromal pigment cell, and potentially resulting in the blue iris color of cormorant species.

Discussion

In this study, we identified the nonsense mutation W49X of *SLC2A11B* responsible for the pigmentation change in the iris of pearl-eyed pigeons. Most of the sampled individuals (141 out of 146) with pearl eyes were homozygous for this mutation, however only five pearl individuals were heterozygous. Possible explanation for these 5 heterozygous pearl eye pigeons is that there might be another mutation in *SLC2A11B* giving rise the same phenotype. Since no other mutation was identified from the *SLC2A11B* coding exons, the additional mutation involve in determining pearl iris color might locate in a totally different gene, or in the regulatory elements of *SLC2A11B* to affect its expression. Alternatively, the phenotype-genotype discordance in these five pearl-eyed individuals carrying one wild-type *SLC2A11B* allele might be due to mis-phenotyping. The iris of newly hatched domestic pigeons was black, and gradually exhibited bright colors after three to four months. Phenotypes of all the pigeons sampled in this study were recorded at about five months old when their iris color is usually turning stable. However, it is likely that the five heterozygous individuals might be younger than the others or there could be individual variance in the growth and development of pigeons whose iris pigmentation change might be still in progress. Unfortunately, we are unable to trace back to these five discordant pigeons to confirm the potential phenotyping error, as they were lost during homing championship in the same year.

The W49X mutation would lead to truncation of about 90% amino acids of the SLC2A11B protein, and was predicted to cause a total loss-of-function in SLC2A11B. In addition to iris, high expression of *SLC2A11B* was also evident in brain, muscle, and retina tissues, suggesting potentially multiple roles of the protein besides its involvement in pigmentation pathways. Therefore, it seems reasonable to expect consequences of the mutation other than iris pigmentation change. However, it seems the influence of *SLC2A11B* mutation in pigeons was restricted to the iris pigmentation, while no other abnormality was apparent in the pearl-eyed individuals. This is an observation consistent with the report that the loss-of-function of *SLC2A11B* in fishes only affected the pigmentation cells (Kimura *et al.,* 2014). It is

likely that the roles of *SLC2A11B* in non-pigmented cells of pearl-eyed pigeons (as well as other *SLC2A11B* mutants) could be compensated by the expression of other genes, mostly likely the other family members of solute carrier family 2.

Although the stromal pigment cell in the pigeon with gravel eyes is generally considered xanthophore-like, it shows a reflecting effect that is normally absent in a typical xanthophore. The stromal pigment cell in pigeons resembles the leucophore in medaka fish, in which the orange pigment is present at the larva stage, but diminishes in the *SLC2A11B*-defect mutant (Kimura *et al.,* 2014). Therefore, it might be more appropriate to refer the pigeon iris stromal pigment cell as leucophore-like. Significantly reduced expression of *CSF1R*, a gene involved in xanthophore differentiation (Kelsh *et al.,* 2010; Patterson *et al.,* 2014; Singh and Nusslein-Volhard, 2015), was detected in the pearl iris (*SLC2A11B* mutant), which indicated that *CSF1R* works downstream of *SLC2A11B* in the regulatory network of xanthophore/leucophore differentiation. In addition, *GCH1*, the gene encoding the first and rate-limiting enzyme of pteridine biosynthesize (Ziegler, 2003; Braasch *et al.,* 2007), was also downregulated in the pearl iris. Since *GCH1* directly participates in the pteridine synthesize, it is plausible that *GCH1* is located in the far end of the regulatory pathway of xanthophore/leucophore differentiation, and triggers the pteridine production upon receiving the upstream signal.

While the precise timing of pigeon domestication is unclear, it is commonly accepted that the pigeon was first domesticated at least 5,000 years ago from the Fertile Crescent (Darwin, 1868; Price, 2002; Driscoll *et al.,* 2009), and probably has served as a food resource for human since as early as 10,000 years ago (Darwin, 1868; Driscoll *et al.,* 2009; Shapiro and Domyan, 2013). The estimated time of origin of *SLC2A11B* W49X mutation at about 5,400 years ago is in line with the commence of pigeon domestication, supporting that the pearl iris trait in domestic pigeons was a derived trait closely associated with the domestication process. Strong signals of positive selection were also detected, providing further evidence that the pearl iris trait has gone through artificial selection.

Even after thousands of years' domestication little morphological difference exists between a wild-type domestic pigeon and its conspecific rock pigeon today, it is reasonable to postulate that the pearl iris could likely serve as one domesticated marker for distinguishing a domestic individual from its wild counterpart during the early stage of domestication. In modern breeds, the reason for the selection of the pearl iris is more complicated. As the eye is one of the classic judging criteria for pigeons, the pearl iris has been preferred in many show breeds such as Show Homer, Runt, and Trumpeter. It is interesting that very high frequency of pearl iris is observed from performance breeds as well, including Tumbler, Roller, and Highflier). Beside a random founder effect, breeders tend to hold an unfounded belief that the pearl eyes in a pigeon is somewhat associated with intelligence and hence better performance.

After birds diverged from their reptile ancestor lineage, one major evolutionary change was the development of the feathery coverage that concealed their skin pigmentation. The function of dermal chromatophores would become redundant, and likely subject to an evolutionary demise. Only epidermal melanocyte retains in modern birds, and plays an important role in feather pigmentation. However, the avian iris seems to maintain the full potential for development of all types of chromatophores, and therefor has been proposed to be a pigment cell "refugium" (Oliphant *et al.,* 1992). The underlying mechanism of this evolutionary change might be that the genes responsible for the development of dermal chromatophores remained intact in the avian genome but showed a tissue-specific expression pattern with high expression in iris tissue while no or only low expression in the skin. Results from this study thus provided some adequate evidence in support of this hypothesis. First of all, *SLC2A11B*, the gene involved in the differentiation of xanthophore and leucophore, is intact in the pigeon genome. And the expression of *SLC2A11B* was high in the iris tissue but extremely low in the skin and feather bud, consistent with the proposed tissue-specific expression pattern. *SLC2A11B* as well as other pigmentation genes in the chromatophore development need to be validated in more avian species to test the hypothesis concerning avian pigment cell evolution.

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Author contributions

X. X. and S. -J. L. conceived the idea for this study. X. X. and designed the study. S. S. and X. X. conducted experiments and bioinformatic analysis. Y. Z. assisted with sampling and technical issues. S.S., X. X. and S. -J. L wrote the manuscript.

Declaration of interests

The authors declare no competing interests.

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Table 1. The validation of *SLC2A11B* W49X mutation in domestic pigeons.

Phenotype	***+/+, W49X/+***	***W49X/W49X***
Yellow	146	0
Pearl	5	141

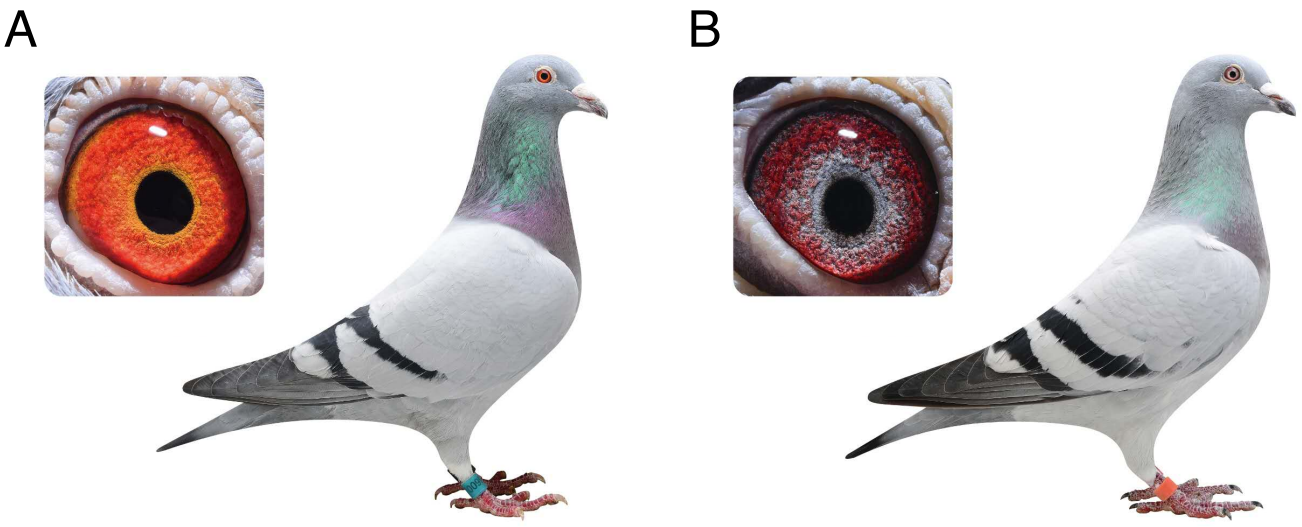

Figure 1. The iris color variations in domestic pigeons. (A) The pigeon with gravel eye (wild-type) exhibits bright orange iris color; (B) The pigeon with pearl eye exhibits greyish white iris color. The red color in both gravel and pearl irises is from blood capillaries.

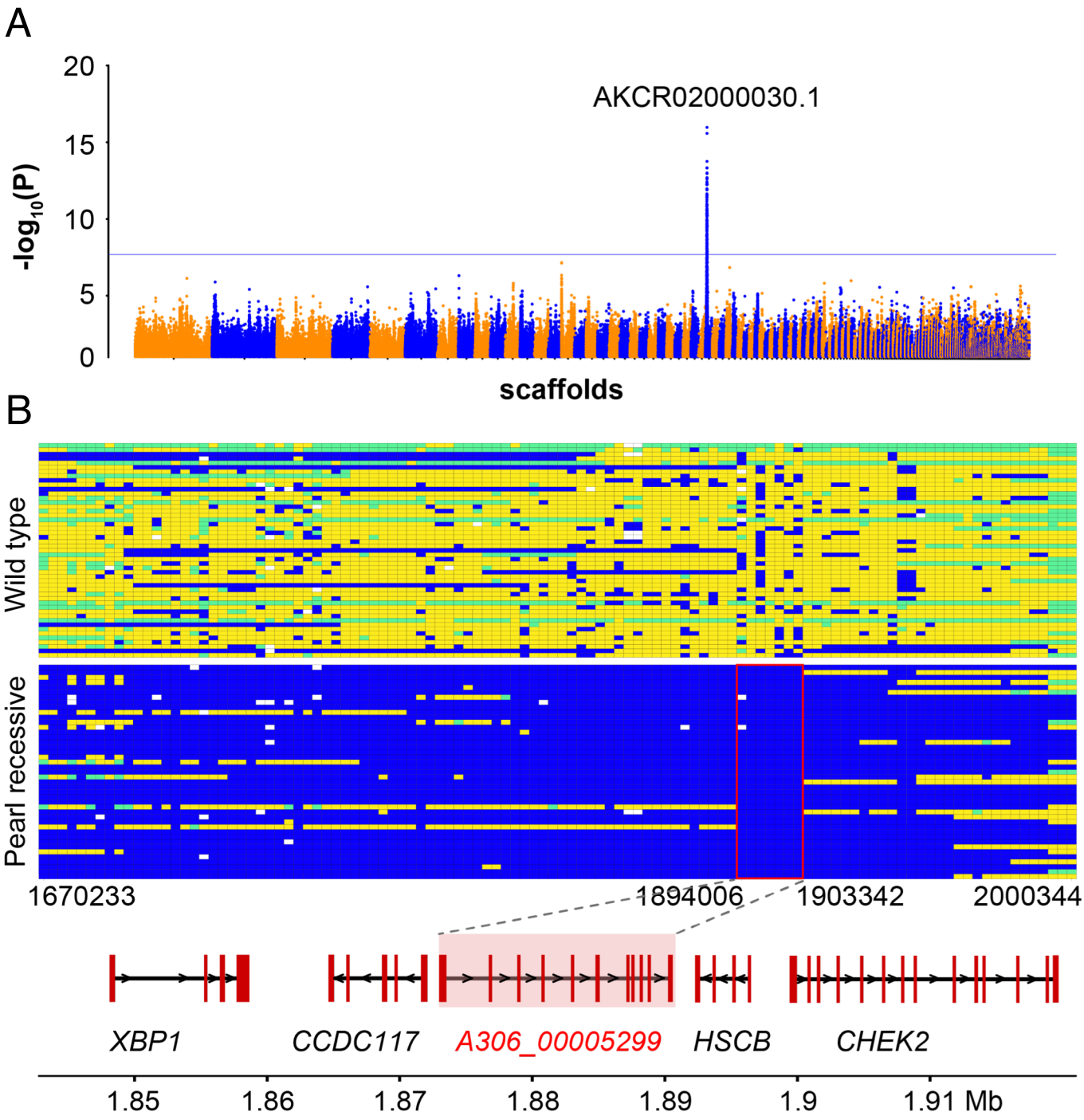

Figure 2. Genetic mapping of the *Tr* locus. (A) The Manhattan plot of the GWAS analysis for the *Tr* locus. The blue line indicates the Bonferroni-corrected critical value (p=2.01E-8). The scaffold with strong association signals is marked. (B) The genotypes of 92 pigeons across the pearl eye associated genomic region. Each row represents an individual, each column represents an SNP/indel, blue represents homozygosity for the major allele in pearl individuals, yellow represents homozygosity for the minor allele in the pearl individuals, green represents heterozygosity, and white means missing data. A shared haplotype (Scaffold 30: 1,894,006-1,903,342) was identified. Genes located in and surround the shared haplotype region is specified.

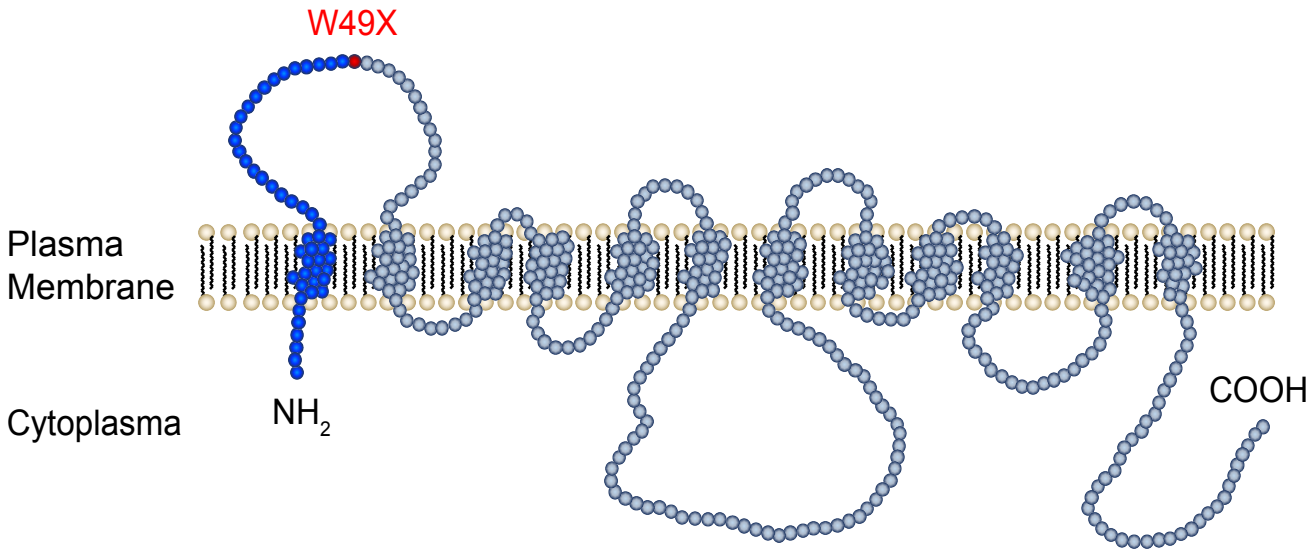

Figure 3. Schematic diagram for the 12 transmembrane SLC2A11B protein. The missense mutation W49X was highlighted in red. Blue circulars presents the remaining amino acids, and grey circulars presents the amino acids truncated by W49X.

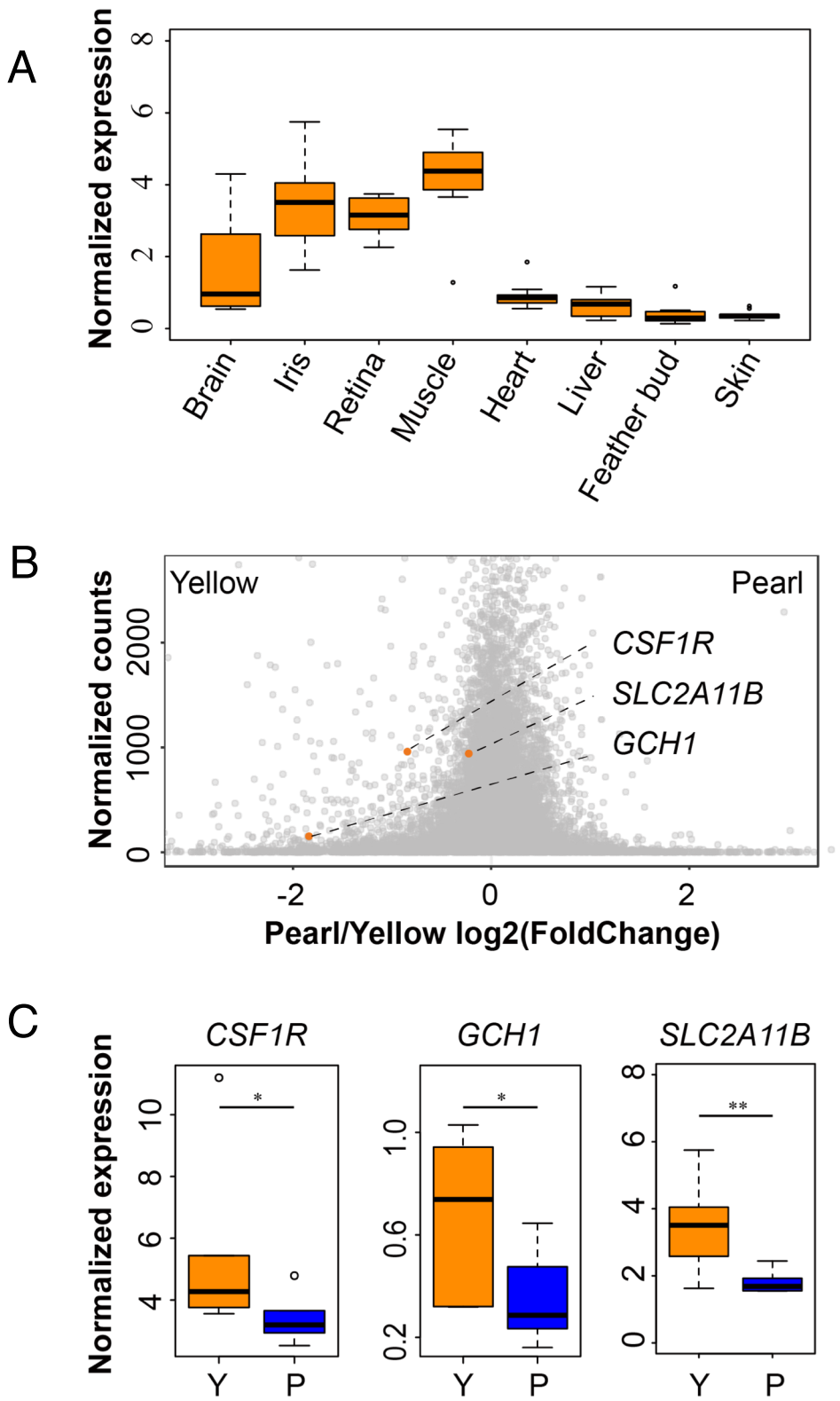

Figure 4. The expression profile of *SLC2A11B* in pigeon tissues and differentially expressed gene between the pearl and gravel irises. (A) Relative expression of *SLC2A11B* in multiple tissues from wild type pigeons by qPCR analysis. Boxes span first to third quartiles, bars extend to minimum and maximum observed values, black line indicates median, and circles represent outlier values. (B) Normalized expression comparison between the pearl and gravel irises by transcriptome sequencing. Differentially expressed genes involved in xanthophore differentiation or pteridine synthesis were highlighted. (C) Validation of differentially expressed genes (*CSF1R, GCH1, SLC2A11B*) between the pearl and gravel irises by qPCR analysis.

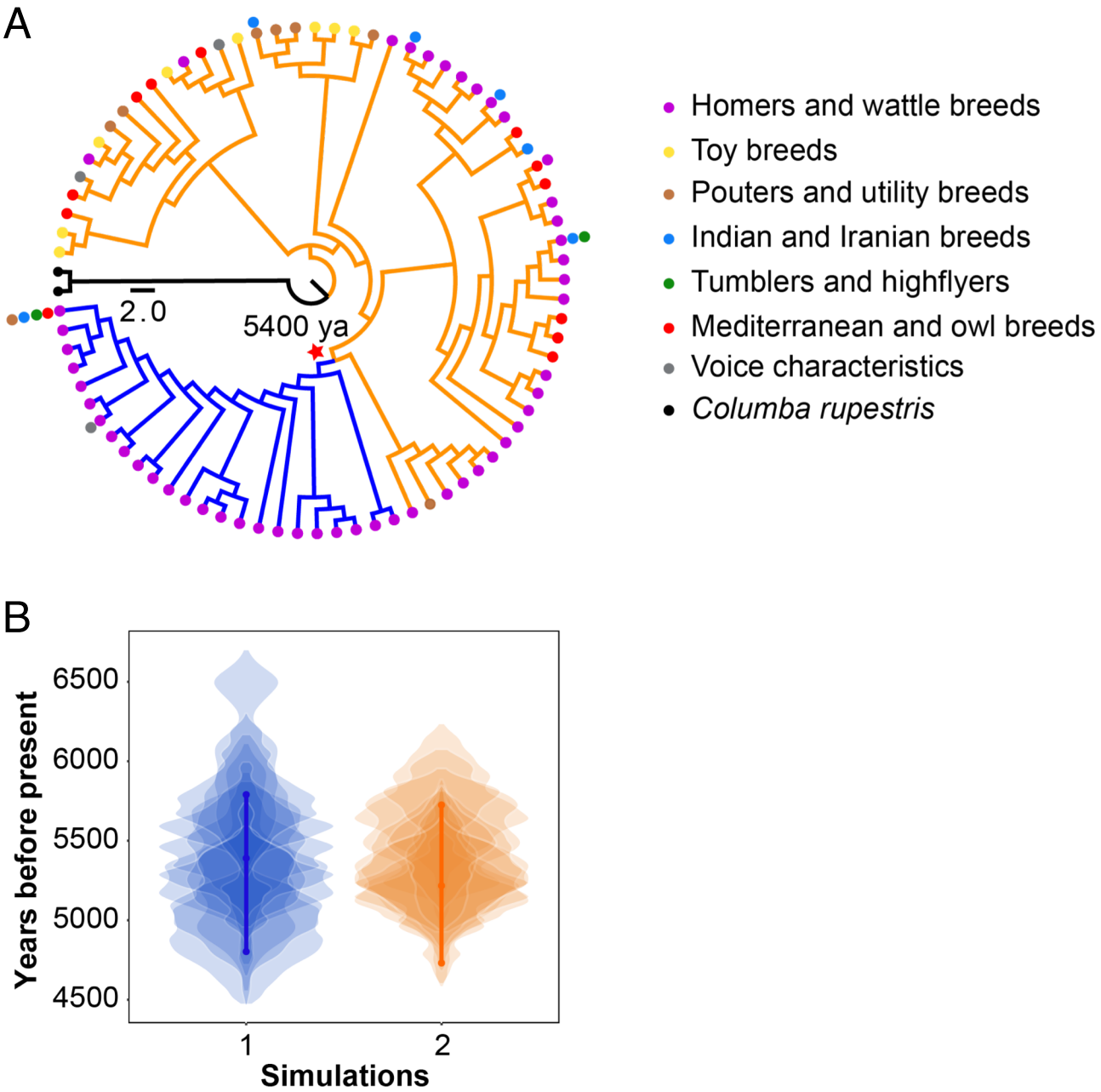

Figure 5. Origin of the *Tr* locus in domestic pigeons. (A) Neighbor-joining (NJ) trees of 9 Kb nonrecombining *Tr* region based on 81 haplotypes from 141 unrelated individuals of 140 domestic pigeons (36 fancy pigeons, 2 feral pigeons, and 102 racing pigeons) and 1 *Columba rupestris* (outgroup). All nodes had more than 60% support from 1000 bootstrap replicates. Blue branches indicate Tr^{mut} haplotypes, orange branches indicate Tr^{wild} haplotypes, black branches indicate the outgroup. Different color of circles represent 7 traditional breed groups of domestic pigeons and outgroup. Red star indicates the origin time of *Tr* mutation. (B) Violin plots of posterior distributions of the estimated TMRCA for *Tr* locus in domestic pigeons with the generation time of one year. Two independent simulation were performed using the estimated recombination rates of 10 Kb and 50 Kb windows, respectively. In every simulation, 10 replicate MCMCs are plotted with transparency.

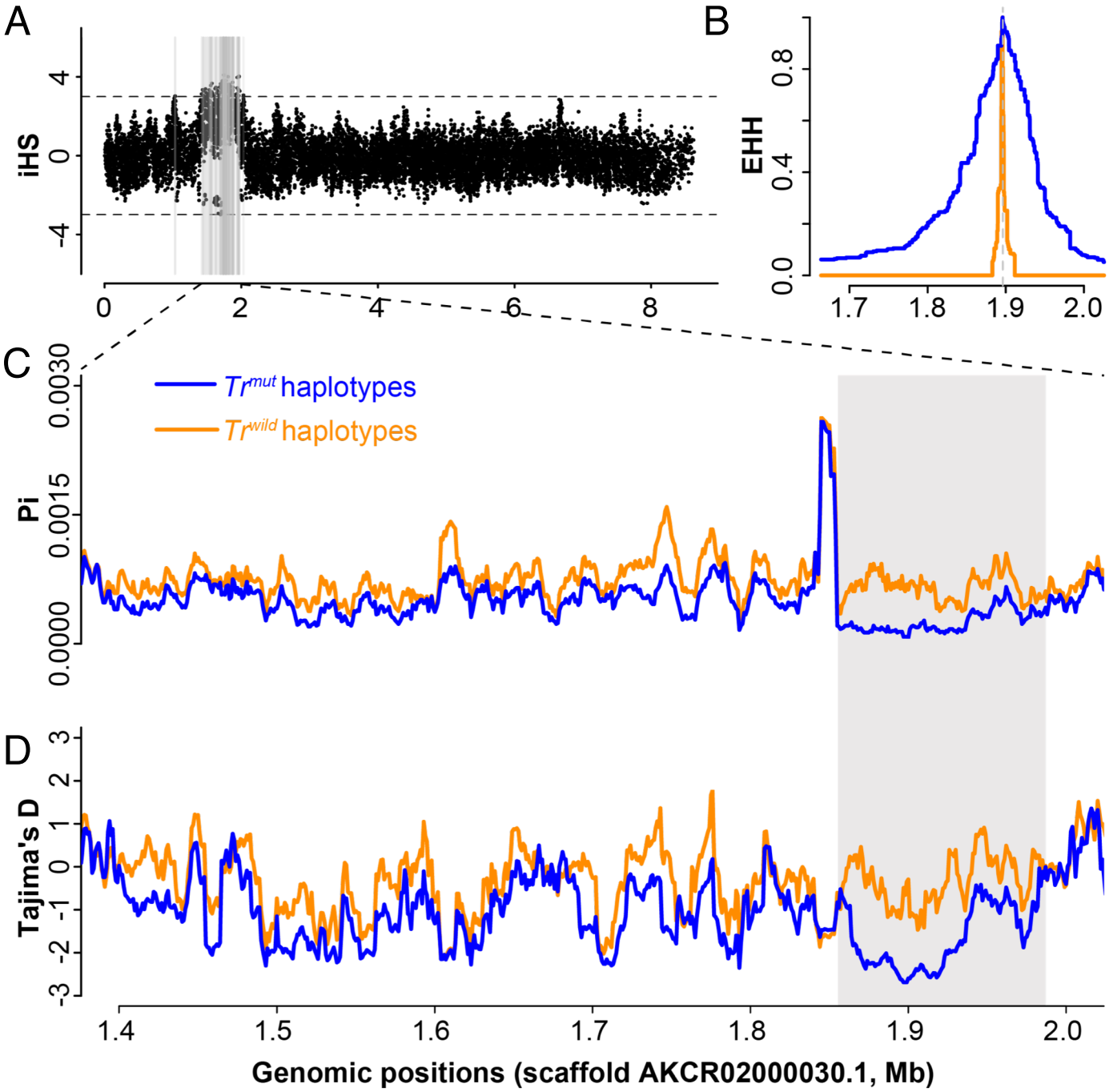

Figure 6. Selection analysis of haplotypes containing W49X mutation (Tr^{mut}) and wild-type haplotypes (Tr^{wild}) surrounding the *Tr* locus. (A) Integrated haplotype score (iHS) was calculated for scaffold AKCR02000030.1 with 1 bp windows. The gray lines represent significance of absolute iHS scores of 3 or greater. (B) Extended haplotype homozygosity (EHH) decay across the 300 Kb *Tr* locus region displayed the increasing distance in Tr^{mut} haplotypes. Nucleotide diversity (π, Pi) (C) and Tajima's D (D) were calculated in 10 Kb windows with 1 Kb step size for Tr^{mut} and Tr^{wild} haplotypes. Blue lines indicates Tr^{mut} haplotypes, orange lines indicates Tr^{wild} haplotypes. Positions on scaffold AKCR02000030.1 are provided. All results were generated using the genomic data of 140 domestic pigeons (36 fancy pigeons, 2 feral pigeons, and 102 racing pigeons).