Knockdown of an ABC transporter leads to bright red eyes in the brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae)

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**A B S T R A C T**

Compound eye color is an important biological character of insects, which is determined by the nature of eye pigments. Ommochrome is the solely source of eye color for some insects, while pteridines is also needed for the other insect species. However, little is known about the eye pigment composition for any planthopper. Scarlet is an ABC (ATP-binding cassette) transporter protein, which functions as the transmembrane transporter for ommochrome precursor. The failure of Scarlet function can cause bright red or white eyes in different species, which depending on the nature of eye pigments. Here, we identified a *scarlet* ortholog gene (*Nlst*) from the brown planthopper (BPH), *Nilaparvata lugens*, which is a destructive insect pest of rice. *Nlst* is the first characterized eye pigment transporter gene from Hemipteran. *Nlst*, the protein deduced from *Nlst* transcript, the wild-type eye color partially changed to bright red, while not to white. Meantime, the ommochrome level in heads reduced to 73.4%. These results suggested that the eye coloration of BPH needs both ommochrome and pteridines pigments. Because nymphal RNAi with *Nlst* leading to a clearly distinguished phenotype from the control individuals, *Nlst* maybe a suitable genetic marker to exploit embryonic RNAi technique in this insect pest.

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**Introduction**

The ATP-binding cassette (ABC) protein family is one of the largest transporter families and is present in all organisms (Dermauw and Van Leeuwen, 2014). The majority of these ABC proteins function as primary-active transporters, requiring the binding and hydrolysis of ATP to transport substrates across lipid membranes (Dermauw and Van Leeuwen, 2014). A functional ABC transporter consists of two cytosolic nucleotide-binding domains (NBDs) that bind and hydrolyze ATP, and two integral transmembrane domains (TMDs). The four domains of a functional transporter can be present in one protein (Full Transporter, FT) or spread over multiple proteins, for example one NBD and one TMD per protein (Half Transporter, HT). HTs need to homo or heterodimerize to form a functional ABC transporter (Higgins, 1992; Higgins and Linton, 2004; Rees et al., 2015).

White, Scarlet and Brown are three HTs, belonging to ABCG subfamily. The proper allocation of eye pigments depends on these three HTs, which import pigment precursors into the appropriate cells for final processing (Dreesen et al., 1988; Tearle et al., 1989; Mackenzie et al., 2000). In *Drosophila*, the White protein forms a heterodimer with Scarlet to transport ommochrome precursors and with Brown for pteridines precursors (Kômoto et al., 2009). Because ommochrome provides brown coloration, failure of Scarlet function results in bright red eyes (Mackenzie et al., 2000), while complete loss of White function means neither pigment source can be transported, so the white-eye phenotype is available (Ewart et al., 1994; Mackenzie et al., 2000). Like in *Drosophila*, mutant or knockdown white orthologs are also the source of the white-eyed phenotype in some insect species (Benedict et al., 1996; Kômoto et al., 2009; Broehan et al., 2013; Grubbs et al., 2015). Unlike in *Drosophila*, mutant white orthologs are not the only factor resulting in white-eyed phenotype in some species. In the silkworm, *Bombyx mori*, mutations in the scarlet ortholog resulted in white eyes (Tatematsu et al., 2011). In the beetle, *Tribolium castaneum*, knockdown of Tcsr also caused a white-eye phenotype (Broehan et al., 2013; Grubbs et al., 2015).

Different phenotype resulted from Scarlet function failure in fruit fly from that in some other insects, like the silkworm and the beetle, indicates that these insects may have varied types of visible eye pigments.

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**Abbreviations:** BPH, brown planthopper; FT, full transporter; HT, half transporter; RACE, rapid amplification of cDNA ends; GSP, gene specific primer; ORF, open reading frame; q RT-PCR, quantitative real-time polymerase chain reaction; NBD, nucleotide-binding domains; TMD, transmembrane domains; Mw, molecular weight; pI, isoelectric point.

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Previous biochemical and microstructure analysis also proved that ommochrome and pteridines were two key forms of insect eye pigments. In the fruit fly, *Drosophila melanogaster* (Summers et al., 1982), or in the grasshopper, *Schistocerca gregaria* (Dong and Friedrich, 2005), ommochrome together with pteridines create wild-type eye color. While ommochrome function as the only pigment source in species as diverse as mosquitoes, moths, bugs, beetles and bees (Dustmann, 1968; Beard et al., 1995; Quan et al., 2002; Moraes et al., 2005; Sethuraman and O’brochta, 2005; Grubbs et al., 2015). As Scarlet is only responsible for ommochrome transport, so function study of Scarlet can be an effective method to speculate the eye pigment source of insects.

The brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), is one destructive pest of rice throughout Asia (Bao et al., 2014; Lin et al., 2016; Xu et al., 2016). The wild-type eye color of BPH is brown, and some red-eye individuals were occasionally observed in lab (Mochida, 1970; Seo et al., 2011; Liu et al., 2014). However, to date, no study has characterized the pigment type of this insect pest or other planthoppers. Here, we cloned and characterized a novel ABCG subfamily gene from *N. lugens* (*Nlst*), which is an ortholog of the *D. melanogaster* scarlet gene. A developmental and body part profile of the *Nlst* gene expression has also been analyzed. Furthermore, knockdown *Nlst* can make the compound eye of this insect pest partially changed to red-eye phenotype, not to white eyes. The present study indicates that the visible pigments of BPH eyes are composed of both ommochrome and pteridines.

Materials and methods

**Insects**

The wild-type planthoppers with brown compound eyes were collected from a laboratory strain. They were reared in light growth chamber under conditions of 27 ± 1 °C, 80 ± 10% RH, and a 16 h: 8 h light: dark photoperiod. In order to obtain synchronized insects, newly hatched nymphs were collected every 12 h and placed into plastic cups containing fresh rice seedlings, which were fixed and moisturized by 1% water agar. The developmental stages were synchronized at each larval molt. In a sample, 50, 40, 30, 20, 10 first- to fifth-instar nymphs, or 8 female/male adults were collected, respectively. Head, thorax, and abdomen were dissected from 3-day-old female adults in cold (–20 °C) and moisturized fixed and moisturized solution (1M PBS, pH 7.0), and immediately frozen in liquid nitrogen for RNA extraction. 50 head, 40 thorax, and 20 abdomen segments were collected in each sample, respectively. For gene fragment clone and rapid amplification of cDNA ends (RACE), a mixture sample was the RNA source, including the first- to fifth-instar nymphs, female and male adults.

**Total RNA isolation and reverse transcription**

Total RNA was extracted using a Trizol kit (Invitrogen). Integrity of the RNA was determined using 1% TAE agarose gel electrophoresis, and RNA was then quantified with a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). First-strand cDNA for RT-PCR was synthesized from 1 μg of total RNA using the reverse transcriptase (M-MLV) with oligo dT18 (Promega, USA). cDNAs for RACE were obtained using SMARTer™ RACE cDNA Amplification kit (Clontech, Mountain View, CA), following the manufacturer instructions. Only the RNA with high quality can be used to generate cDNAs for qRT-PCR. The synthesized first-strand cDNA was used immediately or stored at –20 °C until used.

**Cloning and sequence analysis**

A previously established BPH transcriptome database (1-day-old female adults) was used in this study, which is sequenced on a HiSeq2000 Illumina sequencing platform in BGI-Shenzhen (Shenzhen, China). Raw reads were generated using a Solexa GA Pipeline 1.6 (Illumina). A tblASTN search of this transcriptome database using the *D. melanogaster* Scarlet protein (Genbank number: AAF49455) as queries, revealed one Scarlet-like sequence with 956 bp in length. This sequence was first verified using the primer pairs (Table 1). Full-length cDNA was obtained using RACE method with gene specific primers (GSPs), following manufacturer instructions (Table 1). RT-PCR conditions, cloning and sequencing method were the same as a previous report (Liu et al., 2016b).

**In silico gene sequence analysis**

Gene sequences were assembled and multiple sequences were aligned with DNAMAN 7.0 (Lynnon BioSoft, USA). The open reading frame (ORF) of the target nucleotide sequence was found by the ORF Finder tool at the NCBI website (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). The nucleotide sequence-similarity analyses were performed with the BLAST tool at the NCBI website (https://blast.ncbi.nlm.nih.gov/). The deduced protein sequence was obtained by an ExPaSy translate tool Translate (http://web.expasy.org/translate/), and the calculated isoelectric point (pl) and molecular weight (Mw) were predicted with an ExPaSy proteomics tool Compute pl/Mw (http://ca.expasy.org/tools/pi_tool.html) from the Swiss Institute of Bioinformatics. The N-terminal signal peptide was determined using the SignalP 4.0 server (http://www.cbs.dtu.dk/services/SignalP/). The transmembrane domain and membrane topology was analyzed with TOPCONS online software (http://topcons.cbr.su.se/).

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer sequence (5′ to 3′)†</th>
<th>Sequence length</th>
<th>Description</th>
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<tr>
<td>St-F1</td>
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<td>RT-PCR</td>
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<td>&gt;380</td>
<td>5′ RACE</td>
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<tr>
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<td>&gt;157</td>
<td>3′ RACE</td>
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<tr>
<td>St-T2</td>
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<td>&gt;226</td>
<td>qRT-PCR</td>
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<tr>
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<tr>
<td>RPS15-F</td>
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<td>333</td>
<td>dRNA synthesis</td>
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<tr>
<td>RPS15-R</td>
<td>AGTGGCACTAATTCTCCAGTCC</td>
<td>358</td>
<td></td>
</tr>
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</table>

† The lower case letters in primers are the T7 promoter sequence.
Phylogenetic tree construction

Full-length protein sequences of scarlet genes from different species were retrieved from GenBank. Meantime, we also obtained White and Brown protein sequences from several insect species to conducted sequence alignments with ClustalW using Molecular Evolutionary Genetic Analysis software version 6.0 (MEGA 6). The phylogenetic tree was then constructed using the neighbor-joining (NJ) method with “p-distance” as the amino acid substitution model, “pairwise deletion” as the gaps/missing data treatment, and 1000 bootstrap replications.

Quantitative real-time PCR (qRT-PCR)

qRT-PCR reactions were performed on an ABI 9600 Real-time PCR system (Applied Biosystems, USA) using Power SYBR® Green PCR Master Mix (Applied Biosystems, UK) and GSPs (Table 1). The melting curve and amplification efficiency of every GSP were first determined. Rboosomal protein S15 (RPS15) (Genbank number: ACN79501.1) was used as an internal control, and the primers were the same as Yuan et al. (2014). The PCR reaction volume was 20 μL containing 4 μL diluted cDNA, 0.4 μL of each primer (10 μM/L), 10 μL SYBR Master Mix (2×) and 5.2 μL ddH2O. Two kinds of negative controls were set up including non-template reactions and a reverse transcription negative control. Thermocycling conditions were set as a standard qPCR protocol according to the manufacturer instruction, which were 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 60 s. Only results with single peak in melting curve analyses and >0.95 correlation coefficients were used for subsequent data analysis. Data were analyzed by the 2^−ΔΔCt method (Livak and Schmittgen, 2001). Means and standard errors were obtained from the average of four independent samples, and each sample was repeated in technical duplicate.

dsRNA synthesis and injection

A 333 bp Nlst cDNA fragment was synthesized by PCR, using GSPs incorporating the T7 RNA polymerase promoter sequence (Table 1). This fragment is located in position of 344–454 of the ORF. A previous verified plasmid was used as template. PCR product was purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA) and used for dsRNA synthesis using the T7 Ribomax Express RNAi System (Promega, Madison, WI, USA). The synthesized dsRNAs were respectively isopropanol-precipitated, resuspended in nuclease-free water, quantified by a spectrophotometry (NanoDrop 1000, Thermo Fisher Scientific, USA) at 260 nm, and kept at −80 °C until use.

Fig. 1. Nucleotide and deduced amino acid sequence of the Nlst gene. The stop codon is indicated by an asterisk. The putative polyadenylation signal (AATAAA) is underlined. The seven putative O-glycosylation sites are in brown circles. The feature motif of the ABC transporter gene family is shaded in blue. The two important sequence logos for eye-pigment transporter, the CDEPT motif of the Walker B domain and the IHQP motif of the H-loop, are highlighted in bright yellow. The conserved NBD is underlined in red, and six membrane-spanning α-helices are highlighted in light gray with blue characters. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
use. As a control, a 400 bp enhanced green fluorescent protein (GFP) gene (Genbank number: GQ404376.1) dsRNA was also produced as described above.

Under carbon dioxide anaesthesia, the third-instar nymphs were immobilized on the 1% agarose plate and 50 μL of purified dsRNA (5 μg/μL) was injected following the method of Liu et al. (2010a). The eye color phenotype, ommochrome quantity and mRNA level of Nlst were determined in 4 d after injection.

Pigment analysis

With the same method described by Liu et al. (2014), ommochrome was extracted as follows. Ten heads of each treatment were automatically homogenized in 400 μL of 0.5% ascorbic acid in 1 M HCl. Each homogenate, 400 μL of n-butanol was added in, mixed and then centrifuged at 20000 g for 2 min. Then 200 μL of the upper (butanol) layer was collected and the absorbance measured in a microplate reader (Infiniti M200, TECAN) at 492 nm. n-Butanol was used as the control. Three replicates for each treatment were carried out.

Results

cDNA cloning and characterization

Based on the initial scarlet-like fragment, the full length scarlet homologue cDNA from N. lugens (Nlst) (Genbank number: KU376475) was obtained. The full length Nlst cDNA (2829 bp) contains an ORF of 1890 nucleotides that encodes 629 amino acid residues, and 295- and 644-bp non-coding regions at the 5′-a nd 3′- end of the cDNA, respectively (Fig. 1). At the 3′- end of the Nlst cDNA sequence, a polyadenylation signal sequence AATAAA was apparent upstream of the polyA tail. The Mw and pI of the predicted protein are about 70.60 kDa and 9.11, respectively. Although N-terminal signaling peptides and potential N-glycosylation sites were not found, the deduced amino acid sequence of the Nlst gene (Nlst) contains 7 potential O-glycosylation sites, suggesting that the protein can be glycosylated, which is the same as Pxwhite protein in Plutella xylostella (Guo et al., 2015) (Fig. 1).

In a homology search of GenBank using Blastp tool, the Nlst protein sequence identity with the corresponding Scarlet proteins from other species are all higher than 40%. Nlst exhibits structural features characteristic of known ABC transporter subfamily G, whose members have one NBD with several conserved motifs followed by one TMD with six transmembrane α helix segments at the C-terminus (Fig. 2A and B). Furthermore, the two sequence logos of eye pigment transporters, “CDEPT” in walker B and “IHQP” in H-loop (Grubbs et al., 2015), can also be observed in Nlst protein, which are located in positions 198–202 and 232–235, respectively (Fig. 1).

Phylogenetic analysis of the Nlst gene

Phylogenetic analysis was performed by constructing a neighbor-joining (NJ) tree of Scarlet, White and Brown proteins with MEGA 6.0 based on multiple alignments of amino acid sequences from different species. The unrooted tree showed that the Nlst protein clearly fall into the Scarlet protein group, and it showed clear separation from White and Brown proteins (Fig. 3).

Spatio-temporal expression pattern of the Nlst gene

q RT-PCR study showed that Nlst expressed in all developmental stages (Fig. 4A). Nlst expression peaked in the first-instar nymph and male adults, intermediate in the second-, third- and fourth-instar nymphs and female adults, and lowest in the fifth-instar nymph. In body parts, high level of Nlst transcript was detected in head. Although, Nlst transcript can also be detected in thorax and abdomen, but they were significantly lower than levels in head (Fig. 4B).

RNA interference

To confirm the role of Nlst in eye pigmentation, we performed RNAi by injecting dsRNA of Nlst into the third-instar nymphs, and then the eye-color phenotype and the ommochrome level were evaluated. Fig. 5A showed that injection of dsRNA targeting Nlst reduced Nlst transcript to 13.0% of the control individuals at the fourth day after injection. When Nlst mRNA level was reduced, the compound eye color BPH changed from brown to partially bright red (Fig. 6), and this phenotype

Fig. 2. Structure analysis of the Nlst gene. (A) NCBI conserved domain database (CDD)-based annotation of the Nlst protein. The sequence was identified as a characteristic member of the ABC transporter subfamily G (ABCG) involved in eye pigment precursor transport and pleiotropic drug resistance (EPDR) with 3a01204 as a prediction model. (B) Schematic structure of the Nlst protein. The protein contains six transmembrane segments.
persisted throughout the rest life of the insect. Consistent with the Nlst mRNA level and eye color changing trend, the brown eye pigment, ommochrome, decreased to 73.4% (Fig. 5B) of levels in the control individuals. These results suggested that Nlst also functions to import molecules of the ommochrome pathway.

Discussion

BPH is one destructive insect pest of rice in Asia (Bao et al., 2014; Lin et al., 2016; Xu et al., 2016). The wild-type eye color of BPH is brown, however, several red-eyed mutants have been observed in lab (Mochida, 1970; Seo et al., 2011; Liu et al., 2014; Liu et al., 2015). Like BPH, many eye color mutants have also been described in other insects (Mochida, 1970; Wraight et al., 1999; Shimizu and Kawasaki, 2001; Lorenzen et al., 2002; Snodgrass, 2002; Lohmeyer et al., 2006; Allen, 2013; Wang et al., 2013). As visible genetic marker, eye color mutants can be used for studies on population dynamic monitoring, release and capture studies, mating behavior (Shimizu and Kawasaki, 2001; Khanh et al., 2005; Volkova et al., 2006), fertility and fecundity evaluation (Pires et al., 2002; Snodgrass, 2002), and transgenic and RNAi technology (Fabrick et al., 2004; Dong and Friedrich, 2005; Sethuraman and O’brochta, 2005; Kobayashi et al., 2007; Colinet et al., 2014). Apparently, as a visible genetic marker, the red eye color mutant colony of BPH has great potential for use.

Fig. 3. Unrooted phylogenetic tree of Scarlet proteins and other pigment transport proteins from Insecta species. GenBank accession numbers are displayed within the tree and indicated in parentheses. The scale bar indicates distance in number of substitutions per site. Abbreviations: Aa, Aedes aegypti; Ad, Anopheles darlingi; Ae, Acromyrmex echinatior; Ag, Anopheles gambiæ; An, Apis mellífera; Ap, Acrystosiphon pisum; Bm, B. mori; Bi, Bacrocera tryoni; Cb, Ceracophyys biroi; Cq, Culex quinquefasciatus; Db, Drosophila busckii; Dm, D. melanogaster; Dp, Danaus plexippus; Hs, Harpegnathos saltator; Lc, Lucilia cuprina; Nl, N. lugens; Pm, Papilio machaon; Ps, P. sylvestris; Tc, T. castaneum.

Fig. 4. Relative mRNA expression levels of Nlst at (A) different developmental stages; and (B) different body parts from 3-day-old female adults. First-instar nymph (L1), second-instar nymph (L2), third-instar nymph (L3), fourth-instar nymph (L4), fifth-instar nymph (L5), male adult (MA), female adult (FA), head (H), thorax (T), abdomen (A). The relative expression level was expressed as mean ± SE (N = 3). Different lowercase letters above the columns indicate the significant differences at the P ≤ 0.05 level.
The color of insect eyes is determined largely by the nature of eye pigment (Summers et al., 1982; Ichiki et al., 2007). Tryptophan-derived ommochrome and guanine-derived pteridines are two basic forms of this eye pigment. Ommochrome is the only eye coloration pigment of species as diverse as mosquitoes, moths, bugs and bees (Dustmann, 1968; Beard et al., 1995; Quan et al., 2002; Moraes et al., 2005; Sethuraman and O’brochta, 2005), while in other species, like the grasshopper, S. gregaria (Dong and Friedrich, 2005), or the fruit fly, D. melanogaster (Summers et al., 1982), ommochrome, together with pteridines, create wild-type eye color. However, the pigment source of BPH or other rice planthoppers are yet unclear.

Genes that affect insect eye color often fall into three categories: (i) those that affect ommochrome and/or pteridines biosynthesis; (ii) those that affect transmembrane transport of eye pigments and/or pigment precursors; and (iii) those that affect pigment granule formation (Rasgon and Scott, 2004). For pigment transportation, three half-type ABC transporter proteins, White, Scarlet, and Brown, are responsible for the transmembrane transport of eye pigments and/or pigment precursors (Ewart et al., 1994). The White protein forms a heterodimer with Scarlet to transport ommochrome precursors and with Brown for pteridines precursors (Kômoto et al., 2009). Thus, the mutants of scarlet and brown affect the accumulation of ommochrome and pteridines, respectively, while the white mutants affect the accumulation of both pigments (Ewart et al., 1994).

For some insects, whose eyes are colored with ommochrome and pteridines, the failure of Scarlet function results in bright red eyes, such as the fruit fly (Mackenzie et al., 2000); While for the other insects, whose eyes are colored with solely ommochrome, the failure of Scarlet function causes white eyes, such as the silkworm and the beetle (Tatematsu et al., 2011; Broehan et al., 2013; Grubbs et al., 2015). So, Scarlet provides an excellent marker for analyzing the eye pigment composition of insects.

Here, we cloned the full-length scarlet orthologues gene from N. lugens, which was named with Nlst. Nlst, the deduced protein of Nlst, has high identity with its orthologs in other insect species, and the two sequence logos of eye pigment transporters, “CDEPT” in walker B and “IHQP” in H-loop (Grubbs et al., 2015), were also observed. Phylogenetic analysis also showed that Nlst was closest to those Scarlet proteins from other insects. Nlst is the first eye pigment transporter gene identified from a Hemipteran, although several similar gene fragments have been identified in Lygus hesperus transcriptome (Hull et al., 2014). Apart from Nlst protein, Scarlet/White proteins sequence from Acyrthosiphon pisum have also been available in Genbank database, which benefit from automated computational prediction technology. Expression analysis of the Nlst gene revealed that it expressed in all developmental stages, indicating it is not a timing requirement gene. Nlst temporal expression pattern is consistent with a previous finding of the Dmst gene in D. melanogaster (Tearle et al., 1989). While the high expression of Nlst gene in the last-instar larval (fifth-instar nymph) indicating it may have some correlation with eclosion process, which need further research. Peak expression level of Nlst gene was detected in the head, indicating that the head was the main source of this transcript, which is also compatible with its main function as eye pigment transporter. While expression of Nlst gene in thorax and abdomen reflect that Nlst protein has some other function apart from the one described above. Previous studies have suggested that BmSt is also participated in ommochrome granules deposition in the serosa, and BmSt mutant of B. mori has white eggs (Tatematsu et al., 2011); While knockdown of Scarlet orthologs gene (TcABCG-9A) resulted in white Malpighian tubules in T. castaneum, because the loss of reddish-brown pigments (Broehan et

**Fig. 5.** Knockdown of Nlst transcript and its effect on ommochrome level in heads. (A) The relative expression level of Nlst at 4 d post-RNAi. (B) The relative ommochrome level in heads at 4 d post-RNAi. Control, non-injection; dsGFP, dsRNA targeting GFP; dsSt, dsRNA targeting Nlst. Different lowercase letters above bars indicate significant differences between treatments (P<0.05; Tukey’s test; n = 4).

**Fig. 6.** Effects of reduced Nlst mRNA level on eye pigmentation by RNAi. (A) 4 d after injection with dsGFP; (B) 2 d after injection with dsSt; (C) 4 d after injection with dsSt; (D) 8 d after injection with dsSt. Arrows indicate the red area of compound eye. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
was only partially changed when knocking down
sects also needs both the two types of pigments. Although the eye color
eye coloration of BPH needs both ommochrome and pteridines. This
2011; Broehan et al., 2013; Grubbs et al., 2015), which means that the
however, it is different from that in beetle or silkworm (Tatematsu et al.,
Loukeris et al., 1995; Zwiebel et al., 1995). So, it is not surprising to the existing of
abdomen.
ommochrome quantity, but also partially changed the eye color from
(Allen, M.L., 2013.Genetics of a sex-linked recessive red eye color mutant of the tarnished
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References
Allen, M.L., 2011. Genetics of a sex-linked recessive red eye color mutant of the tarnished
serine protease gene family and expression profile analysis in the planthopper,
Nilaparvata lugens. BMC Genomics 15, 1.
Anopheles gambiae pink-eye and white genes define distinct, tightly linked eye-color loci. J. Hered. 87, 48–53.
Broehan, G., Kroege, T., Lorenzen, M., Merzendorfer, H., 2013. Functional analysis of the
ATP-binding cassette (ABC) transporter gene family of Tribolium castaneum. BMC Genomics 14, 6.
a genetic marker for fertility and fecundity of Tribolium castaneum (Klug, 1834) at the
Dernaewe, W., Van Leeuwen, T., 2014. The ABC gene family in arthropods: comparative
melanogaster is similar to the White protein and to components of active transport complexes. Mol. Cell. Biol. 8, 5206–5215.


