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An orange-eye mutant of the white backed planthopper, *Sogatella furcifera* (Hemiptera: Delphacidae)

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Abstract: 【Aim】 This study aims to investigate the gene controlling the eye color of the white-backed planthopper (WBPH), *Sogatella furcifera* and to explore the potential influence of eye color mutants on mating capacity. 【Methods】 An orange-eye mutant was established in our insectary, and the gene symbol related to this mutant was designated as *org*. The genetic basis of this phenotype was determined by designing eight groups of cross-mating experiment containing three genotypes (+/+, +/*org*, *org*/*org*). Whether the eye color related genes influence the mating capacity in WBPH was tested in a mating competition experiment. 【Results】 This orange-eye mutant had a pair of bright orange ommatea which was distinctly different from the wild type WBPH. The orange-eye phenotype occurred in all developmental stages of WBPH: the eyespot of eggs (3 d after ovipisition) and the compound eyes of nymphs and adults of both sexes and wing morphs. Reciprocal crosses between homozygous normal-eye WBPH and orange-eye WBPH produced F_1 progeny with only normal-eye color. The sex ratio in the offspring produced from all groups was not biased to a specific sex based on *Chi*-square test ($\alpha = 0.05$, $\chi_c^2 = 0.19 - 1.53$). The mating competitiveness of the orange-eye mutant was significantly weaker than that of the wild type phenotype in WBPH. 【Conclusion】 Our results indicate that the inheritance of the orange-eye trait is controlled by an autosomal recessive allele, and eye color related gene has significant impacts on mating capacity in WBPH.

Key words: *Sogatella furcifera*; eye color mutant; orange-eye; autosomal recessive allele; hybridization experiment; mating competition

1 INTRODUCTION

White backed planthopper (WBPH), *Sogatella furcifera*, as one of the most harmful migrant pests of *Oryza sativa*, has attracted much attention in Southeast Asia. WBPH damages rice by nymph and adult piercing-sucking (Liu *et al.*, 2005), immigrant, and transmission of the virus (Wang *et al.*, 2005). The compound eyes of insects are the primary receptors to sense light and changes of surrounding environment. It is highly ordered by hundreds of functional units, named ommatidia (Mishra and Knust, 2013). Crystalline cone and rhabdome are enclosed by pigment cells (Stumm-Tegethoff and Dicke, 1974). Inheritance law of the insect compound eye research covers Diptera (Saul and McCombs, 1992; Yamada and Selivon, 2001; Rasgon and Scott, 2004), Hemiptera (Snodgrass, 2002; Lohmeyer *et al.*, 2006; Seo *et al.*, 2011), Coleoptera (Lorenzen *et al.*, 2002) and Lepidoptera (Rananavare *et al.*, 1989). Some eye color related genes were located on autosome, the others were sex-linked (Al-Hakkak *et al.*, 1985; Rasgon and Scott 2004; Seo *et al.*, 2011). Researches on *Triatoma infestans* Klug (Hemiptera, Reduviidae) showed that the gene controlling eye color did not influence the mating attraction, but the sperm cells from red-eye mutants have lower survivability than those from the wild types (Pires *et al.*, 2002). In addition, previous studies proved that the level of xanthommatin and pteridine in pigment cells determined the color of compound eyes (Liu *et al.*, 2014), and could be used as a visible genetic marker in studying functional genomics.

In the previous study, a red-eye mutant has been reported in *Laodelphax striatellus* (Fallén) (Ishii, 1966). In addition, a red-eye mutant and an orange-eye mutant have been reported in two isolates of *Nilaparvata lugens* (Stål), respectively (Mochida, 1970; Seo *et al.*, 2010). All the mutant phenotypes were controlled by single autosomal recessive gene in the three mutants. However, no

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eye color mutant was reported in WBPH.

We established an orange-eye mutant strain and further applied a cross-mating experiment to prove the mode of inheritance of orange color in compound eye and the potential of which could be used as a visible genetic marker in studying functional genome. Then we performed a mating competition experiment to reveal whether there is any difference in mating attraction between orange-eye mutant ones and normal-eye ones.

2 MATERIALS AND METHODS

2.1 Establishment of WBPH colony

WBPHs used in this study were established from a male adult mutant with orange-eye which was found in greenhouse at Zhejiang University, Hangzhou, Zhejiang, China. The gene symbol related to this mutant was designated as *org*. We collected this original mutant and fed on fresh rice seedlings (variety: Xiushui134, the same as the original host) in a transparent plastic jars (8 cm in diameter, and 10 cm in height) and mixed with 10 wild type normal-eye female WBPHs in the same incubator (25 ± 1℃, RH 65% ± 5% and a photoperiod of 16L: 8D) in order to preserve the rare gene. The progenies reproduced by them were all normal-eye. Next F_1 individuals were reared under the same conditions. Among F_2 individuals, there were two kinds of eye color. One was normal-eye, the other was orange-eye (Fig. 1). The normal-eye WBPHs usually have crimson eyes during the egg and early nymphal stages, and the compound eyes become dark yellow or all black in the 5th instar nymphal and adult stage. While the orange eyes occur in all stages of orange-eye mutant from egg to adult regardless of sex, wing type, or body color. We designed a cross-mating experiment to explore the rule of eye color inheritance in WBPH. In this series of experiment, the host rice was 5 cm tall and planted in nutrient solution with pledget covered the root. Each clump of rice was about 2.5 cm apart.

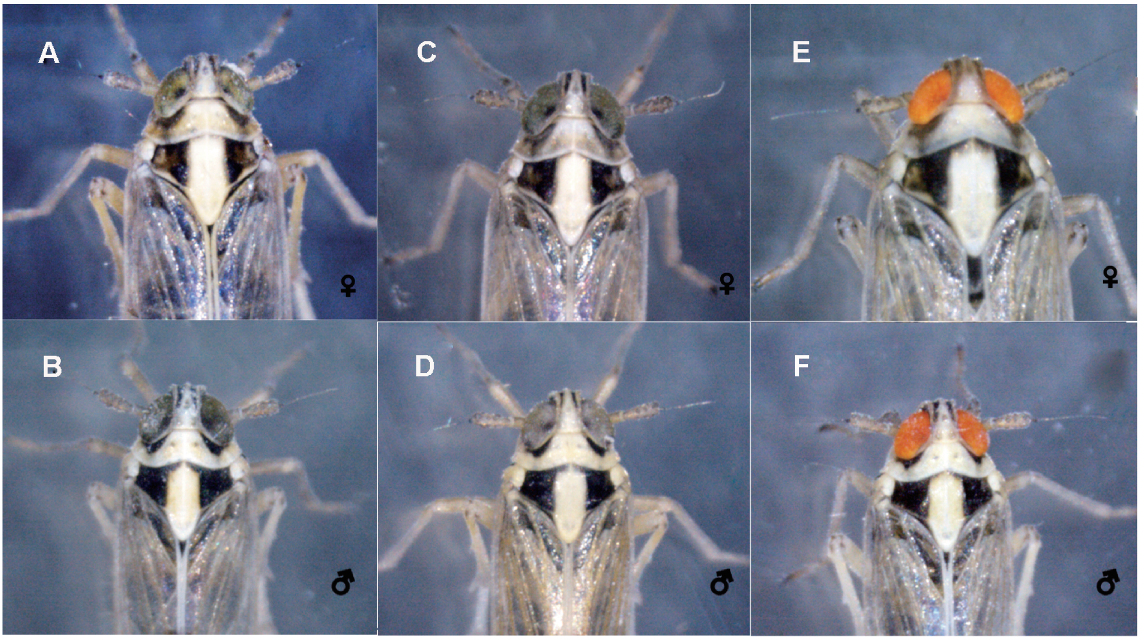

Fig. 1 Photographs of compound eyes in three genotypes of *Sogatella furcifera* adults under stereoscopic microscope
A: Homozygous normal-eye, ♀; B: Homozygous normal-eye, ♂; C: Heterozygote, ♀; D: Heterozygote, ♂; E: Homozygous orange-eye, ♀; F: Homozygous orange-eye, ♂. Scale bars = 1 mm.

2.2 Hybridization

Hereditary trait of the orange-eye was revealed by reciprocal crosses in two phenotypes of WBPHs (the normal-eye WBPH and the orange-eye WBPH) which were the isogenic lines whose genetic background was purified through inbreeding for five generations. Back-crosses and each combination of F_1 inbreeding were carried out. Before the hybridization experiment, the male nymphs at the 5th instar stage were separated from the females of the same instar by morphology and fed independently in different transparent plastic jars (8 cm in diameter, and 10 cm in height) with rice seedlings. Eclosion occurred about 4 d after the 5th instar nymphal stage. After emergence, the male and females were immediately reared separately in plastic jars to ensure that they were unmated. In this experiment, we used the same wing type of adults.

After 3 d, we mixed one female and one male corresponding to the combination designed before:

F: Normal (+/+) × M: Normal (+/+);
F: Orange (*org/org*) × M: Orange (*org/org*);
F: Orange (*org/org*) × M: Normal (+/+);
F: Normal (+/+) × M: Orange (*org/org*);
F: Normal (+/*org*) × M: Orange (*org/org*);
F: Orange (*org/org*) × M: Normal (+/*org*);
F: Normal (+/*org*) × M: Normal (+/*org*);
F: Normal (+/*org*) × M: Normal (+/+).

Each couple was transferred to new jars everyday till the female was dead. Five to seventeen repetitions were set in each group respectively (Table 1). Then we used the random sampling method for data statistics. Theoretically, the orange-eye phenotype was controlled by a single autosomal recessive allele. The result of allozygotes showed that there is no significant difference between the orange-eye phenotype and the heterozygotes. Theoretically, the two phenotypes would be separated in F_2: the expected ratios of normal-eye and orange-eye would be 3:1 in F_2 and 1:1 in back-cross progenies.

2.3 Mating competition

We designed a mating competition experiment to explore whether there was significant difference in WBPH mating competitiveness between normal and mutant. Three WBPHs (female orange-eye, male orange-eye and male normal-eye) were placed in the same incubator at 3 d after their sexual maturity. Through visual observation, the males would compete with each other and finish mating in half an hour. The whole mating process lasted about 1 min. The females usually mate only once during their lifetime. After mating, the females were picked out and waited for the birth of F_1. This experiment was replicated 15 times. Among the 15 replicates, females in 14 replicates laid eggs, female in one replicate was dead. Creating a fair competition condition in mating competition experiment is an important process, where the steps might directly affect the accuracy of the results. The following steps help to make it convincing. (1) Rice seeds used for accelerating germination were cleaned before soaking. (2) Selection of each adult was finished within 5 h after eclosion. It usually takes some time to wait for the maturity of gonads. It was essential to keep adults from mating at this time point. (3) Different wing morphs might affect the result. In this research, all the WBPHs are long-winged morphs. (4) The WBPHs are mixed in random for the purpose of simulating the natural environment.

2.4 Data analysis

The hybridization data were analyzed by testing the goodness of fit of the expected phenotypic ratio and sex ratio in each phenotype. The aim of χ^2 tests for the goodness of fit is to determine whether the expected ratio has significant difference from the observed ratio. The χ^2 could be calculated by the formula below:

$$\chi_c^2 = \sum_{i=1}^{N} \frac{(|O_i - E_i| - 0.5)^2}{E_i}$$

N is the number of phenotypes; i is the ith observed or expected value; O is the observed value in each phenotype; E is the expected value in each phenotype; 0.5 is a correction constant for continuity. Compared calculated χ_c^2 with $\chi^2_{(0.05,1)}$ in *Chi*-square distribution table, if $\chi_c^2 < \chi^2_{(0.05,1)}$, we could conclude that the observed ratio has no significant difference from the expected ratio.

3 RESULTS

3.1 Orange-eye mutant controlled by autosomal recessive gene

The mode of inheritance of the orange-eye phenotype in WBPH was presented in Table 1. Number of pairs, different phenotypes in progeny including nymph and adult were all listed. Reciprocal crosses between homozygous normal-eye WBPH and orange-eye WBPH produced F_1 progeny with only normal-eye color (Table 1, groups 3 and 4). Back-crosses between the heterozygous F_1 WBPH and the homozygous orange-eye WBPH produced the offspring with normal-eye color and orange-eye color at 1:1 ratio (Table 1, groups 5 and 6). Inbreeding between the heterozygous F_1 progeny resulted in F_2 offspring with normal-eye color and orange-eye color at the 3:1 ratio (Table 1, group 7). The χ_c^2 of groups 5, 6 and 7 were all less than $\chi^2_{(0.05,1)} = 3.84$, meaning that the observed eye color ratio had no significant difference from the expected ratio. Back-crosses between the heterozygous F_1 WBPH and the homozygous normal-eye WBPH resulted in the offspring with only normal-eye color (Table 1, group 8). The sex ratio in the offspring produced from all groups was not biased to a specific sex based on *Chi*-square test ($\alpha = 0.05$). The χ_c^2 value ranged from 0.19 to 1.53, which were much less than $\chi^2_{(0.05,1)} = 3.84$ (Table 1). These results suggested that the inheritance of the orange-eye phenotype was controlled by a single autosomal recessive allele, but not the sex chromosome, according to the Mendel's law.

3.2 Mating capacity of orange-eye mutant

In the mating competition experiment, except for group 1, F_1 in other groups were all normal-eye

Table 1　Inheritance of the orange- and normal-eye phenotypes in *Sogatella furcifera*

Group	Copulation		Number of pairs	Expected ratio (N∶O)	Statistics of phenotype in the offspring									
	Female	Male			Nymph				Adult				Expected sex ratio of orange-eye (♀∶♂ =1∶1) *Chi*-square ($\chi^2_{(0.05,\,1)}$)	Expected sex ratio of normal-eye (♀∶♂ =1∶1) *Chi*-square ($\chi^2_{(0.05,\,1)}$)
	Phenotype (genotype)	Phenotype (genotype)			Normal	Orange	Observed ratio (N∶O)	*Chi*-square ($\chi^2_{(0.05,\,1)}$)	O♀	O♂	N♀	N♂		
1	Normal (+/+)	Normal (+/+)	5	1∶0	625	0	/	/	0	0	135	157	/	1.51
2	Orange (*org*/*org*)	Orange (*org*/*org*)	17	1∶0	0	1 327	/	/	213	199	0	0	0.41	/
3	Orange (*org*/*org*)	Normal (+/+)	5	0∶1	380	0	/	/	0	0	152	140	/	0.41
4	Normal (+/+)	Orange (*org*/*org*)	6	0∶1	729	0	/	/	0	0	167	181	/	0.49
5	Normal (+/*org*)	Orange (*org*/*org*)	14	1∶1	922	982	0.94∶1	1.83	257	232	241	270	1.18	1.53
6	Orange (*org*/*org*)	Normal (+/*org*)	7	1∶1	323	297	1.09∶1	1.01	78	90	100	93	0.72	0.19
7	Normal (+/*org*)	Normal (+/*org*)	7	1∶3	612	189	3.24	0.77	55	62	136	156	0.31	1.24
8	Normal (+/*org*)	Normal (+/+)	5	0∶1	677	0	/	/	0	0	160	172	/	0.36

Not all progenies from each cross mating were observed for the sex ratio in adult stage. O is orange, and N is normal. /: Not observed.

Table 2 Mating competition between normal-eye phenotype and orange-eye mutant in *Sogatella furcifera*

Group no.	Parent phenotype and number	Eye color of F_1 nymph	
		Normal	Orange
1	♀: Orange × 1 ♂: Normal × 1 Orange × 1	31	45
2	♀: Orange × 1 ♂: Normal × 1 Orange × 1	106	0
3	♀: Orange × 1 ♂: Normal × 1 Orange × 1	125	0
4	♀: Orange × 1 ♂: Normal × 1 Orange × 1	149	0
5	♀: Orange × 1 ♂: Normal × 1 Orange × 1	71	0
6	♀: Orange × 1 ♂: Normal × 1 Orange × 1	96	0
7	♀: Orange × 1 ♂: Normal × 1 Orange × 1	124	0
8	♀: Orange × 1 ♂: Normal × 1 Orange × 1	133	0
9	♀: Orange × 1 ♂: Normal × 1 Orange × 1	123	0
10	♀: Orange × 1 ♂: Normal × 1 Orange × 1	93	0
11	♀: Orange × 1 ♂: Normal × 1 Orange × 1	103	0
12	♀: Orange × 1 ♂: Normal × 1 Orange × 1	59	0
13	♀: Orange × 1 ♂: Normal × 1 Orange × 1	100	0
14	♀: Orange × 1 ♂: Normal × 1 Orange × 1	102	0

(Table 2), which suggested that the mating competitiveness of the orange-eye mutant was significantly weaker than that of the normal-eye phenotype in WBPH. Observations showed that nearly all the groups finished copulation in 10 min. It also indicated that female individuals wouldn't mate with another male during their lifetime after the first copulation. This result may suggest that the eye color related gene may influence the mating capacity of WBPH.

4 DISCUSSION

Our results suggested that the inheritance of the orange-eye phenotype was controlled by a single autosomal recessive allele, but not the sex chromosome. For other hopper species, the genes controlling eye color are also located on autosome. Previous study showed that eye color mutant appearing in *N. lugens* (Hemiptera: Delphacidae) (Liu *et al.*, 2014) and *L. striatellus* (Hemiptera: Delphacidae) (Wang *et al.*, 2013) were controlled by an autosomal recessive allele.

In the previous study, the eye color related gene was also showed to affect the mating ability of the small brown planthopper, *L. striatellus* (Wang *et al.*, 2013). Our results from mating capacity were consistent with that of the small brown planthopper, indicating that there are some underlying metabolic pathways that affect both eye color and mating ability. This pleiotropy effect is of great importance for investigation, and helpful for the further studies on eye color mutant.

Eye color phenotype in most insects was controlled by a single autosomal recessive allele, except in some of dipteral insects whose eye color phenotype was related with sex chromosome. Previous studies showed that few eye color mutation may have lethality to nymph (Mochida, 1970). In our study, eye color mutation had no obvious lethality to nymph or showed matrilineal inheritance. There were four types of mutation which affected the eye color, *i.e.*, ommochrome, pteridine biosynthesis, transmembrane transport of eye pigments (or precursors) and pigment granule (Rasgon and Scott, 2004). It was known that xanthommatin was one of four kinds of ommochrome, and was resulted from the product of tryptophan metabolic pathway: tryptophan → kynurenine → 3-OH-kynurenine → xanthommatin. If one of the enzymes was blocked, the components of pigment would change so that the eye color would also change. Further studies of the genetic and metabolic basis of the eye color related gene in WBPH would provide deeper insights into this topic. In addition, there was correlation between kynurenine metabolic pathway with longevity and age-related diseases (Lorenzen *et al.*, 2002). Several hypotheses could explain this pleiotropy effect. First, the eye color related genes are also involved in the pathways which would affect mating capacity. Second, there exist non-random mating patterns for WBPH, as WBPHs prefer to mating with others with specific phenotypes or genotypes. Third, the eye color related genes and mating related genes are in linkage disequilibrium and they are more likely to co-inherit when reproducing. Further studies should figure out the genetic basis of these phenomena.

Eye color related genes were very important molecular genetic markers, which would promote the construction of transgenic system and RNAi of nymph system. They would also be useful in the analysis of migratory flight and mating behavior (Liu *et al.*, 2014).

Based on these, establishing an independent transgenic system, and overexpressing certain genes may provide insights into functional genome in WBPH. However, it should be noticed when using the orange-eye mutation as marker for functional genomics studies, the mutation shows negative effects on male mating competition.

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一种白背飞虱橙眼突变体

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摘要：【目的】本实验的目的是研究白背飞虱 *Sogatella furcifera* 控制不同眼色的基因以及眼色突变对交配能力的潜在影响。【方法】在实验室中构建了一种新型的白背飞虱橙色复眼突变体，与此性状相关的基因型符号为 *org*。利用包含 3 种基因型（+/+，+/*org*，*org*/*org*）的 8 组杂交组合实验来确定影响橙眼性状的遗传基础，并通过一项交配竞争实验来测试复眼眼色突变是否对白背飞虱交配能力有影响。【结果】该种橙眼突变体具有一对与野生型白背飞虱有明显区别的亮橙色复眼。在突变体白背飞虱发育阶段均出现了橙眼性状：卵期眼点（产卵 3 d 后出现），若虫复眼和任意性别翅型的成虫复眼。纯合子正常眼白背飞虱和橙眼白背飞虱之间的互交产生了 F_1 正常眼颜色的后代。卡方检验表明交配后代并未倾向于特定的性别（$\alpha = 0.05$，$\chi_c^2 = 0.19 \sim 1.53$）。橙眼白背飞虱突变体的交配竞争能力显著弱于野生型白背飞虱。【结论】橙眼性状由常染色体隐性基因控制，且控制眼色的 *org* 基因对交配竞争能力有显著影响。

关键词：白背飞虱；眼色突变；橙眼；常染色体隐性基因；杂交试验；交配竞争

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