



An orange-eye mutant of the brown planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae)

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ABSTRACT

An orange-eye mutant of the brown planthopper (BPH), *Nilaparvata lugens* (Stål), was found in a green house and has since been maintained together with a normal-eye phenotype of BPH in an insectary. The orange color was expressed in all developmental stages of BPH: the eye spots of eggs and the eyes of nymphs and adults of both sexes and wing forms. Cross-mating results suggested that the inheritance of the orange-eye phenotype is controlled by a single autosomal recessive allele. The gene symbol related to this mutant was designated as “org”. Developmental duration and mortality of nymphal stages were not significantly different between the normal phenotype (homozygous and heterozygous) and the mutant. In addition, reproduction was not significantly different among mating combinations of the three BPH genotypes (+/+, +/-org, org/org). The effect of eye color on mating of BPH was insignificant in a mate choice test which consisted of one orange-eye female, one orange-eye male, and one homozygous normal-eye male. Offspring produced by the orange-eye female BPH hatched and developed into adults normally, indicating that the eye color mutant found in this study is different from the red-eye BPH (Mochida, 1970) which showed the egg lethal effect in the red-eye BPH female.

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Introduction

Eye color mutants are commonly found in many insect species. Many researchers have studied their genetic inheritance and physiological effects (e.g. viability, fecundity) (Spencer, 1928; Al-Hakkak et al., 1985; Taylor and Cuevas, 1986; Ranavavare et al., 1989; Shimizu and Kawasaki, 2001; Snodgrass, 2002; Rasgon and Scott, 2004; Ichiki et al., 2007), biochemical and molecular mechanisms of pigmentation regulation (Hiraga, 1964; Summers and Howells, 1978; Puckett and Petty, 1980; Summers et al., 1982; White et al., 1996; Lorenzen et al., 2002; Rasgon and Scott, 2004; Moraes et al., 2005), and behavior (mating attraction, mating frequency, and competition) (Shimizu and Kawasaki, 2001; Pires et al., 2002). In addition, eye color mutants and their related genes have recently been studied as potential genetic markers for transformation in various insects (White et al., 1996; Lorenzen et al., 2002; Yan et al., 2008).

In Delphacidae (Hemiptera), red-eye mutants were reported in the small brown planthopper, *Laodelphax striatellus* (Fallén) (Ishii, 1966) and the brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Mochida, 1970). Mutants were controlled by single autosomal recessive gene in both. A new mutant form of BPH with orange-

colored eyes was found on rice plants in a greenhouse in Suwon, Republic of Korea. Here, we report the mode of genetic inheritance of the orange-eye phenotype in BPH and its fundamental biological characters.

Materials and methods

Experimental population of BPH

An orange-eye BPH mutant was found in the greenhouse of National Institute of Crop Science (NICS), Suwon in May 2006. Several macropterous females and males and nymphs of the orange-eye BPH mutant were collected and allowed to mix with the normal-eye, macropterous BPH colony (2005-BPH, Seo et al., 2010) in a rearing cage (W30×L50×H25 cm). The mixed colony was maintained on Ilpumbyeo seedlings (a *japonica* type rice cultivar). BPH rearing and all experiments were carried out at 25 ± 2 °C, 60 ± 5% RH and 15L:9D photoperiod.

The normal type BPH usually has dark-red eyes during the egg and early nymphal stages. The compound eyes are dark yellow in the fifth nymphal stage and the adult stage. In the orange-eye BPH, orange eyes occur in all developmental stages from egg to adult regardless of sex, wing form, and body color (Fig. 1). This mutant was named as “orange-eye BPH” based on the yellowish red eye color in bright light.

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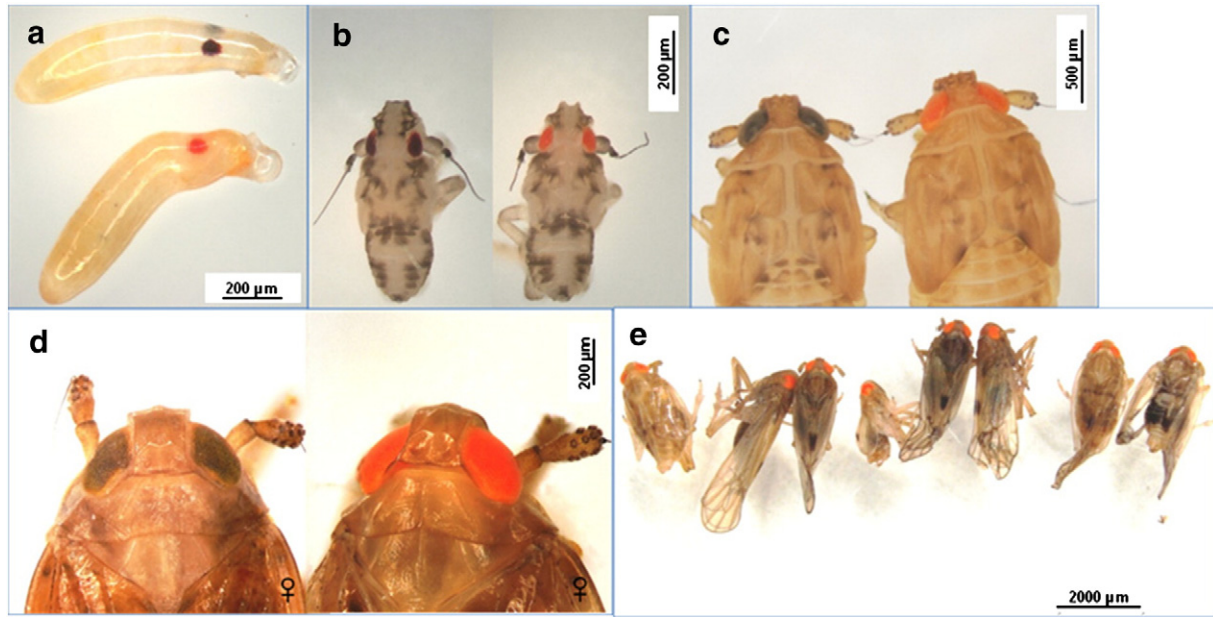


Fig. 1. Photographs of a normal-eye BPH (a, above; b, left; c, left; d, left) and an orange-eye BPH (a, below; b, right; c, right; d, right; e) in different developmental stages. a: Egg, b: first instar nymph, c: fifth instar nymph, d and e: adult.

Cross-mating experiment

Inheritance of orange-eye phenotype was examined through reciprocal crosses between normal- and orange-eye BPHs, several combinations of F_1 inbreedings, and back-crosses. For each cross, unmated females and males were prepared by isolating fifth instar nymphs in glass tubes (3 cm diameter, 20 cm height) containing fresh rice seedlings. When adults emerged, one male/female pair was introduced into a glass tube with a fresh rice seedling. Each male/female pair was transferred to a new tube every day until the

female died. Seedlings with eggs were kept until the eggs hatched. The phenotype was determined immediately after hatching and their sexes were determined in adult stage. In the case that the orange-eye phenotype was controlled by a single autosomal recessive allele, it was expected that ratios for the F_2 and back-cross progenies would be 3:1 and 1:1 (normal-eye to orange-eye), respectively, and the ratio of female to male offspring in each eye color phenotype would be 1:1. The gene symbol related to the orange-eye mutant was designated as “org”. Data were analyzed by χ^2 tests for the goodness of fit of the expected phenotypic ratio and sex ratio in each phenotype.

Table 1
The mode of inheritance of the orange-eye phenotype in BPH.

Cross	Mating		No. of pairs	Expected ratio of the theoretical segregation		Observed phenotype of progeny									
	Female	Male		Orange	Normal	Nymph			Adult ^a						
	Phenotype (genotype)	Phenotype (genotype)				Total no. of progeny	Orange	Normal	Chi-square ($\chi^2_{0.05, 1}$)	O♀	O♂	N♀	N♂	Expected sex ratio of orange-eye (♀:♂ = 1:1)	Expected sex ratio of normal-eye (♀:♂ = 1:1)
														Chi-square ($\chi^2_{0.05, 1}$)	Chi-square ($\chi^2_{0.05, 1}$)
1	Orange (org/org)	Orange (org/org)	12	1	0	1030	1030	0		74	97	0	0	3.09	
2	Orange (org/org)	Normal (+/org)	2	1	1	187	101	86	1.20	5	9	6	13	1.14	2.58
3	Orange (org/org)	Normal (+/+)	8	0	1	641	0	641		0	0	171	186		0.63
4	Normal (+/+)	Orange (org/org)	4	0	1	486	0	486		0	0	174	133		5.48 ^b
5	Normal (+/org)	Orange (org/org)	7	1	1	1595	777	818	1.05	263	251	254	293	0.28	2.78
6	Normal (+/org)	Normal (+/org)	7	1	3	1681	441	1240	1.37	133	127	317	359	0.14	2.61
7	Normal (+/org)	Normal (+/+)	2	0	1	213	0	213		0	0	– ^c	–		
8	Normal (+/+)	Normal (+/+)	14	0	1	2087	0	2087		0	0	540	629		6.78 ^b

^a Not all progenies from each cross mating were observed about the sex ratio in adult stage (O: orange-eye, N: normal-eye).

^b Indicates that the sex ratio was significantly different from 1:1 (cross 4, $P=0.02$; cross 8, $P=0.01$).

^c Not observed.

Table 2
Developmental period and survival rate of the nymphal stages of homozygous BPHs and heterozygous BPH.

Cross ♀ × ♂	Phenotype and genotype of F1 progeny	n	Nymphal duration (days, mean ± sd)						Survival rate (%) ^a during nymphal stage
			1st stadium	2nd stadium	3rd stadium	4th stadium	5th stadium	Total	
Normal (+/+) × normal (+/+)	Normal (+/+)	60	3.03 ± 0.18	2.24 ± 0.43	2.61 ± 0.49	2.78 ± 0.42	3.85 ± 0.69	14.57 ± 0.60	96.7
Orange (org/org) × normal (+/+)	Normal (+/org)	60	3.12 ± 0.42	2.12 ± 0.45	2.20 ± 0.44 ^b	2.76 ± 0.50	4.05 ± 0.76	14.32 ± 0.98	95.0
Orange (org/org) × orange (org/org)	Orange (org/org)	60	2.92 ± 0.33	2.45 ± 0.53 ^b	2.30 ± 0.50 ^b	2.65 ± 0.55	4.40 ± 0.73 ^b	14.74 ± 1.04	95.0

^a Nymphal survival rates were not significantly different among three different genotypes by the log-rank test and the Wilcoxon test at the 95% confidence level (log-rank, $P=0.73$; Wilcoxon, $P=0.96$).

^b Indicates that there is significant difference in comparison with normal (+/+) BPH by Dunnett's t -Tests ($\alpha=0.05$).

Comparison of biological characters between mutant and normal BPH

The developmental duration and survival rate of nymphal stages were compared among the three genotypic groups (org/org: orange eye, +/org: normal eye, +/+: normal eye). Sixty neonate nymphs of each genotype were incubated individually in a glass tube (3 cm diameter, 20 cm height) containing young rice seedlings. Exuvium casts and mortality were checked every day. Dunnett's t -Tests ($\alpha=0.05$) for nymphal duration and the log-rank test and the Wilcoxon test ($\alpha=0.05$) for survival rates were used to compare among the three genotypes. Reproductive ability was measured as the number of nymphs hatched. The results from several mating combinations (Table 1) were compared with those from the homozygous (+/+) normal BPH parents by Dunnett's t -Tests ($\alpha=0.05$). Data analysis was conducted by SAS Enterprise Guide 4.2 (SAS Institute, 2008).

The effect of eye color on mating of BPH

To understand the effect of orange eye color on mating, we designed a mate choice test that consisted of three one-day old BPH adults: one orange eye female, one orange eye male, and one homozygous normal eye male. In total, ten replicates were performed. Each mating pair was kept in a glass tube (3 cm diameter, 20 cm height) containing a young rice seedling and transferred to a new tube every day until the death of the female. Seedlings with eggs were kept in BPH rearing conditions and the number of hatched-nymphs with normal or orange eye phenotype was counted every day. After most nymphs hatched, the color of the eye-spots in the remaining eggs was also checked. If an orange eye female mated with a homozygous normal eye male and/or an orange eye male, heterozygous normal eye and/or orange eye progeny were expected. The expected ratio between the two possible mating cases, (orange eye ♀ × homozygous normal eye ♂) vs. (orange eye ♀ × orange eye ♂), was 1:1. Mate choice among mutant female, mutant male and homozygous normal male was statistically compared by chi-square test ($\chi^2_{0.05, 1}$).

Results and discussion

Reciprocal cross-mating between homozygous normal-eye BPH and orange-eye BPH produced F1 offspring with only normal-eye color (Table 1, crosses 3 and 4). Inbreeding between the heterozygous F1 progeny resulted in F2 offspring with normal-eye color and orange-eye color at 3:1 ratio (Table 1, cross 6). Back-crosses between the heterozygous F1 BPH and the homozygous orange-eye BPH produced the offspring with normal-eye color and orange-eye color at 1:1 ratio (Table 1, crosses 2 and 5). Back-crosses between the heterozygous F1 BPH and the homozygous normal-eye BPH resulted in offspring with only normal-eye color (Table 1, cross 7). The sex ratio in the offspring produced from most crosses was not biased to a specific sex based on chi-square test ($\alpha=0.05$). Although only the progenies from cross 4 ($\chi^2_{0.05, 1}=5.48, P=0.02$) and cross 8 ($\chi^2_{0.05, 1}=6.78, P=0.01$) were not equal in the sex ratio, the ratio of female to male was 1.31 and 0.86, respectively, which were close to 1 (Table 1). These results

suggest that the inheritance of the orange-eye phenotype is controlled by a single autosomal recessive allele according to the Mendel's law, and is not in the sex chromosome.

There were significant differences in the developmental durations of nymphal stages among the three genotypes by F -tests (1st: $F_{2, 177}=5.73; P=0.0039$, 2nd: $F_{2, 176}=7.57; P=0.0007$, 3rd: $F_{2, 176}=11.88; P<0.0001$, 4th: $F_{2, 174}=1.17; P=0.3138$, 5th: $F_{2, 171}=8.67; P=0.0003$) (Table 2). The duration of the third nymphal stage was significantly reduced in heterozygous normal-eye phenotype and homozygous orange-eye phenotype compared to the homozygous normal-eye phenotype (Dunnett's t -Tests, $\alpha=0.05$). The second and the fifth nymphal durations of the homozygous orange-eye phenotype were significantly longer (Dunnett's t -Tests, $\alpha=0.05$). However, the total duration of the nymphal stage was not significantly different between the normal-eye phenotype (homozygous and heterozygous) and the orange-eye phenotype (Dunnett's t -Tests, $\alpha=0.05$). In addition, the nymphal survival rates were not significantly different among the three genotypes (log-rank test, $P=0.73$; Wilcoxon test, $P=0.96$) (Table 2).

The number of progeny of several mating combinations of the three genotypes was not significantly different from that produced by homozygous normal-eye BPH pairs (Dunnett's t -Tests, $\alpha=0.05$), suggesting that there is little or no egg lethal effect (Fig. 2).

The red-eye BPH showed egg lethal effects in the blastokinesis stage within the ovaries of the red-eye female, resulting in no progeny from any mating combination with the red-eye female BPH (Mochida, 1970). In the red-eye mutant of *L. striatellus*, reduced fecundity was observed compared to the normal-eye SBPH (Ishii, 1966). In the orange-eye BPH, however, it seems that there is little or no egg lethal effect. The mean number of progeny of the normal-eye BPH parents ((+/+) × (+/+)) was higher and was not significantly different than

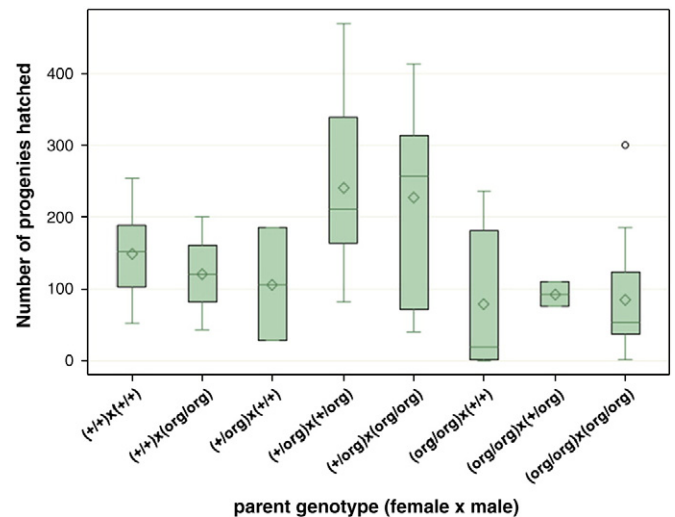


Fig. 2. Reproductive ability of several mating combinations of three different BPH genotypes (+/+, +/org, and org/org). The number of progeny in 14 pairs of normal-eye BPH (+/+) was compared with those in other pairs of BPHs. There was no significant difference in reproductive ability among mating combinations of three different BPH genotypes (Dunnett's t -Tests, $\alpha=0.05$).

Table 3
Number of mating pairs and their F1 progeny resulting from a mate choice test which consisted of one orange-eye BPH female, one orange-eye BPH male, and one homozygous normal-eye BPH male.

Mating combination	Mating type ^a	Number of mating pairs	Number of F1 progeny produced		Chi-square test ^b
			Orange-eye (org/org)	Normal-eye (+/org)	
Orange eye (org/org) ♀ × orange eye (org/org) ♂ × normal eye (+/+) ♂	A Normal-eye progenies only	4	0	1270	0.14 (P=0.71)
	B Orange-eye progenies only	3	663	0	
	C Normal-eye progenies first, and later orange-eye progenies	2	190	415	
	D Orange-eye progenies first, and later normal-eye progenies	1	134	64	

^a According to the distribution of the eye-color phenotype of F1 progeny and their hatching-order in each mating pair, four mating types could be distinguished in this test. Mating type A and B showed only one eye-color phenotype, normal and orange, respectively, and type C and D produced both eye-color phenotypes in a different hatching-order.

^b Expected ratio on the total number of the two possible mating cases, (orange eye ♀ × normal eye ♂) and (orange eye ♀ × orange eye ♂), was 1:1 and statistically compared by chi-square test ($\chi^2_{0.05, 1}$).

those of the orange-eye female BPH couples, ((org/org) × (org/org)), ((org/org) × (+/org)), and ((org/org) × (+/+)) (Fig. 2). Furthermore, the maximum number of progeny produced by the normal-eye BPH and the orange-eye BPH parent was 254 and 301, respectively. This suggests that the reproductive ability of the orange-eye BPH is not reduced at all compared to that of the normal-eye BPH.

On the other hand, Ishii (1966) and Mochida (1970) reported that nymphal duration and survival rate were not significantly different between the normal-eye phenotype and the red-eye mutant phenotype. The total developmental period and survival rate of the orange-eye BPH nymphs and the heterozygous normal-eye BPH nymphs were also not significantly different compared with those of the homozygous normal-eye BPH nymphs (Table 2).

In the results of the mate choice experiments (Table 3), four mating pairs (type A) produced only normal-eye BPH offspring, while three mating pairs (type B) produced only orange-eye BPH. This ratio was not deviated to either type A or type B by chi-square test ($P=0.71$), suggesting that the effect of orange-colored eyes on mating was insignificant. Interestingly, both phenotypes were produced in the remaining three mating pairs. But, in two mating pairs (type C) out of three, only the normal-eye nymphs hatched at first and the orange-eye nymphs started to hatch from the 13th and the 27th rice seedlings, respectively. The remaining mating pair (type D) produced offspring in the reverse order. After only the orange-eye nymphs hatched continuously from the rice seedlings in the early mating period, the normal-eye nymphs started to hatch from the 14th rice seedling. These results indicate that female BPH could have multiple matings with several males and that there may be more complex interactions in BPH mating than expected.

The color of the insect compound eye is determined mainly by the nature of the screening pigments, ommochrome (brown, derived from tryptophan) and/or pteridine (red, derived from guanine), which are deposited in granules located in the primary and secondary pigment cells of the ommatidia (Summers et al., 1982). There are four general mutant categories which affect the color of the insect compound eye: (1) that in ommochrome biosynthesis, (2) that in pteridine biosynthesis, (3) that in transmembrane transport of eye pigments and/or precursors, and (4) that in pigment granule formation (Rasgon and Scott, 2004). In *do* (dark-orange), an eye-color mutant of the housefly, *Musca domestica* L., the amount of tryptophan was 5 times higher than in wild type, but, accumulations of kynurenine and 3 OH-kynurenine were similar (Hiraga, 1964). It was also found that enzyme activity of over-all conversion of tryptophan to kynurenine in the cell-free extract of this mutant was half that of the wild type, suggesting that the dark-orange eye of this mutant is related to blocking the early step of ommochrome biosynthesis. *Drosophila* eye-color mutants, *vermillion* (*v*) and *cinnabar* (*cn*), are also involved in the ommochrome biosynthesis (*v* gene – tryptophan oxygenase; *cn* gene – kynurenine 3-mono-oxygenase) (Lorenzen et al., 2002). Ommochrome pigments are commonly found in the compound eye of insects, while pteridine pigments are mainly limited to the Diptera

(Lorenzen et al., 2002). From these reports, it can be inferred that the screening pigments of the orange-eye BPH are highly affected by a certain step in ommochrome biosynthesis. The biochemical and molecular mechanisms of eye pigmentation regulation related to the orange-eye BPH will be studied in the near future.

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