A New Report of *Nezara viridula* f. *aurantiaca* (Heteroptera: Pentatomidae) from a Cultured Population in Washington County, Mississippi

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Abstract

*Nezara viridula* adult coloration can vary, including a rare orange-colored type (i.e., *N. viridula* f. *aurantiaca*). In November 2015, three *Nezara viridula* males displaying orange coloration were found in an established colony in Stoneville, MS. The objectives of this study were to determine if alleles of these orange types conformed to the allele characteristics previously reported for *N. viridula* f. *aurantiaca* and to determine if there were any differences in reproductive output compared with the green-colored type. The three orange-type males were crossed with green-type females to produce a hybrid F1. The F1 progeny was allowed to cross to produce an F2. The F2 progeny consisted of 672 green females, 351 green males, 298 orange males, and 0 orange females. These ratios did not differ significantly from the expected 50:25:25:0 ratios for a single recessive sex linked allele for color phenotype. The F2 cross of green females and orange males produced an F3 consisting of 345 green females, 346 green males, 100 orange females, and 85 orange males. These ratios also conformed to the expected ratios (0.375:0.375:0.125:0.125) with the exception of orange males, which numbers were slightly lower than expected. The pure orange type *N. viridula* produced significantly less egg masses (0.71 ± 0.15) per day than green types (2.09 ± 0.16) and their reproductive output, measured as net reproductive rate (R0), was lower in orange (13.71) compared with green (20.67) types.

Key words: Southern green stink bug, orange type, coloration, sex-linked, recessive allele

The southern green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae), is a worldwide polyphagous pest that damages economically important crops such as soybeans (*Glycine max* (L.)), green beans (*Phaseolus vulgaris* L.), black-eyed peas (*Vigna unguiculata* (L.)), cotton (*Gossypium hirsutum* L.), and peanuts (*Arachis hypogaea* L.) (Hirose et al. 2006). *Nezara viridula* adults are reported to present body color polymorphism from black to orange (Esquivel et al. 2015). The rare uniformly orange-colored morph (*N. viridula* f. *aurantiaca*) has been reported in Brazil, Japan, and Hawaii from field collections (Yukawa and Kiritani 1965; Vivan and Panizzi 2002; Follett et al. 2007); only one specimen was collected from field samples in Homestead, FL, and College Station, TX (Esquivel et al. 2015). *Nezara viridula* coloration is controlled by a single sex-linked gene, where the orange allele is recessive to the green allele (Follett et al. 2007). In November 2015, three *N. viridula* males with orange coloration were observed in a colony maintained at the USDA-ARS National Biological Control Laboratory in Stoneville, MS. The objectives of this study were to determine whether alleles of the observed orange types conformed to the allele characteristics previously reported for *N. viridula* f. *aurantiaca* and to compare the reproductive output of green versus orange type pure colonies.

Materials and Methods

A colony was established from soybean and cowpea field collections of *N. viridula* in Stoneville, MS, during the spring and the summer of 2010. This colony has been maintained in culture for 5 years at the USDA-ARS National Biological Control Laboratory. After approximately 40 generations in culture, three orange morph adult males were detected in the colony.

The orange morph males were paired with green females at a two females per male sex ratio to represent a parent population (F0), and allowed to reproduce using the rearing methods described by Rojas and Morales-Ramos (2014). Cages constructed from clear plastic boxes (*L* 320 × *W* 260 × *H* 100 mm, Part no. 048-C, Pioneer Packing, Dixon, KY) were modified with ten windows (27 mm dia.) on the sides and four windows (65 mm dia.) on the top all covered with nylon screen (mesh 500 μm). All adults in this study were reared in an
environmentally controlled room at 26 ± 1 °C, 50 ± 5% RH, and 14 h photophase and provisioned with peanuts, broccoli, a diet supplement, and fresh bean pods and a diet supplement that was replaced at 3 days intervals. Eggs were collected daily and hatching nymphs (F1) were allowed to develop under same conditions. All emerging adults were sexed and counted. The number of males and females of each coloration type was recorded for each generation in this study.

Resulting F1 adults were paired and reared at the same conditions to obtain the F2 generation. Resulting F2 adults consisted of green females and green and orange males. Only F2 orange males (approx. 25% of the total numbers) were retained to mate with F2 females (at two females per male sex ratio) to produce the F3. Eggs were collected daily and enclosing nymphs were reared to the adult stage under the same conditions described above. Because the F3 colony had become considerably larger, only resulting F3 adults from the first three emerging dates were counted and sexed. The number of males and females of each coloration type was recorded for each generation in this study.

Results and Discussion

The F1 generation resulted in only green morphs (590 ♀ and 684 ♂) as expected from a recessive orange allele of single gene character. However, the F2 generation produced 672 green females, 351 green males, 298 orange males, and no orange females. These ratios were

| Table 1. Expected progeny ratios and observed frequencies in resulting F1, F2, and F3 generations after cross of orange males and green females in parent generation (F0). |
|---------------------------------------------------------------|
| Generation: parent and (progeny) | Assumed Genotypes | Expected phenotype ratios in progeny | Observed phenotype frequencies in progeny | X² |
|----------------------------------|------------------|-------------------------------------|-------------------------------------------|-----|
| F0 (F1) X G♂G♂ x X♂♂Y            | G♂ G♂ O♂ O♂     | 0.5 0.5 0 0                       | 590 684 0 0                              | 6.936 |
| F1 (F2) X G♂G♂ x X♂♂Y            | G♂ G♂ O♂ O♂     | 0.5 0.25 0 0.25                   | 672 351 0 298                           | 4.653 |
| F2 (F3) a 0.5 X G♂G♂ x X♂♂Y + 0.5 X G♂G♂ x X♂♂Y | G♂ G♂ O♂ O♂     | 0.375 0.375 0.125 0.125           | 345 346 100 85                          | 8.067* |
| Degrees of freedom (df) = 3; X² values with "*" show significant deviation from expected ratios. |
| aF2 green males were removed leaving only orange males to cross with green females. |

Fig. 1. Cumulative lxmx (reproductive output) from fertility tables of green and pure orange types of Nezara viridula. The end of the lines provides the R0 values for each color type.
not significantly different to those expected for a recessive allele of a sex-linked gene (Table 1) and were consistent with Follett et al. (2007). The first three cohorts of the F3 generation consisted of 345 green females, 346 green males, 100 orange females, and 85 orange males. The difference between observed and expected ratios were significant ($\chi^2 = 8.067, \text{df} = 3, P = 0.046$) (Table 1), but this difference could be explained by significant deviations from the expected sex ratio of the F3 orange types ($|Z| = 2.38, P = 0.0087$) resulting in a slight bias of 1.17 females per male. A similar female bias sex ratio was observed by Follett et al. (2007) and this bias was explained as higher mortality in males occurring during their development.

Mean oviposition rate ($\pm \text{SEM}$) measured as eggs per female per day was significantly higher in green-type stock colony females ($2.76 \pm 0.29$) as compared with F3 orange-type females ($1.17 \pm 0.27$) ($|T| = 2.456; \text{df} = 222; P = 0.0148$). There was no significant difference in the number of eggs per egg mass between the parental stock green ($27.14 \pm 1.26$) and F3 orange ($26.39 \pm 1.62$) types. Also, the proportion of fertile eggs was not significantly different between green ($0.718 \pm 0.0094$) and orange ($0.726 \pm 0.0058$) types. However, green-type females produced significantly higher number of egg masses per day ($2.09 \pm 0.16$) than orange type females ($0.71 \pm 0.15$) ($|T| = 6.14; \text{df} = 222; P < 0.0001$). The reproductive output of green-type females was higher as measured by the $R_o$ value ($20.67$) compared with orange-type females ($13.71$) (Fig. 1). In addition to the reduced expected phenotypic ratio in natural populations (consisting mostly of males due to the sex linked and recessive nature of the orange allele), the lower reproductive output by orange types may be an explanation for their rarity in natural populations.

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References Cited

Esquivel, J. F., V. A. Brown, R. B. Harvey, and R. E. Droleskey. 2015. A black color morph of adult Nezara viridula (L.). Southwest. Entomol. 40: 648–652.

Follett, P. A., F. Calvert, and M. Golden. 2007. Genetic studies using the orange body color type of Nezara viridula (Hemiptera: Pentatomidae): inheritance, sperm precedence, and disassortative mating. Ann. Entomol. Soc. Am. 100: 433–438.

Hirose, E., A. R. Panizzi, and A. J. Cattelan. 2006. Potential use of antibiotic to improve performance of laboratory-reared Nezara viridula (L.) (Heteroptera: Pentatomidae). Neotrop. Entomol. 35: 279–281.

Portilla, M., J. A. Morales-Ramos, M. G. Rojas, and C. A. Blanco. 2014. Life tables as tools of evaluation and quality control for arthropod mass production, pp. 241-75. In J. A. Morales-Ramos, M. G. Rojas, and D. I. Shapiro-Ilan (eds.), Mass production of beneficial organisms, invertebrates and entomopathogens. Academic Press, Waltham, MA.

Rojas, M. G., and J. A. Morales-Ramos. 2014. Juvenile coloration as predictor of health in Nezara viridula (Heteroptera: Pentatomidae). J. Entomol. Sci. 49: 166–175.

Vivan, L. M., and A. R. Panizzi. 2002. Two new morphs of the southern green stink bug, Nezara viridula (L.) (Heteroptera: Pentatomidae), in Brazil. Neotrop. Entomol. 31: 475–476.

Yukawa, J., and K. Kiritani. 1965. Polymorphism in the southern green stink bug. Pac. Insects. 7: 693–642.