Image Analysis of Intra-Population Variability in a Non-Outbreak Population of the Rice Black Bug *Scotinophara* sp. from Tacurong, Sultan Kudarat, Philippines

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**Abstract** - Rice black bugs (RBB) are believed to be a serious pest of rice in the Philippines. The identification and classification of RBB in the Philippines has been a problem because of immense variability thus this study was conducted to describe the extent of variability in wing venation patterns and genital shapes in a non-outbreak population of the insect. Images of the forewings were taken and the venation patterns were traced using Adobe Photoshop for analysis and comparison. Landmark-based Geometric morphometric (GM) analyses of the genital plates were also done. Results showed interindividual disparity in the wing venation patterns with marginal cells observed to vary in number. The longitudinal veins were seen as either forked or continuous towards the distal margin of the wings. On the other hand, GM analyses showed that the genital plates varied with respect to the lengthwidth aspect ratio, concavity of the anterior margins and shapes of the anterior and posterior lateral margins. The observed variability in morphological features is discussed in relation to density-dependent phase polyphenism.

**Keywords** - genitalia, geometric morphometrics, Phenotypic plasticity, wings

**I. INTRODUCTION**

Phenotypic plasticity is a common characteristic among insects which has the ability of its genotype to exhibit variable phenotypes in such variable environment (Pfennig et al., 2010). Phenotypic plasticity can be adaptive (improving organism’s survival and reproduction) in this case the insect genitalia. But not all phenotypic plasticity is adaptive that includes typically the wings of insects because of its biochemical, physiological and developmental biology which considered them to be plastic (Pigliucci et al., 2006).

In most cases, wings are considered an important organ of insects for it can adapt to various environmental conditions (Cui, 2010). Wing traits are evolving rapidly in respond to artificial selection and geographic clines (Gilchris et al., 2000; Huey et al., 2000; Houle et al., 2003; Kennington et al., 2003). Wing venation pattern and wing shape is speciesspecific thus it is used in taxonomic identification (Tofilski, 2004). Another important organ of insects which is adaptive as stated earlier is the genitalia. It is traditionally been assumed that the rate of evolutionary change in secondary sexual characters among species exceeds with that of non-sexual characters giving rise to a high degree of divergence (Cuervo & Møller, 2006). Precise species identification is feasible through examining the external morphological characteristics and the genital traits which can show intrapopulation and
inter-population variation (de Freitas & Hermanson, 2007). Understanding variability in wing venational patterns and external genitalia in a non-outbreak population of the Rice Black Bug *Scotinophara* spp. is of great importance in assessing the extent of variability of this insect pest of rice. Knowledge of how such variability maybe of immense importance in the formulation of strategies geared towards the management and control of the insect pest which is considered one of the major pests of rice in the Philippines.

Population density can affect the amount of phenotypic diversity in populations of pests. This phenomenon, aptly called density-dependent phase polyphenism is extensively studied in the migratory locust, *Locusta migratora*, where individuals in outbreak populations are said to differ much in terms of morphology and behaviour (Chapuis et al., 2008). Phenotypic diversity is produced when different rearing densities during juvenile stages generates individuals that vary in a variety of traits including morphology (Chapuis et al., 2008; Jannot et al., 2009). This phenomenon assumes that populations in low non-outbreak densities are phenotypically depauperate when compared to outbreak populations. Thus, this study determined the level of phenotypic diversity in a non-outbreak population of rice black bugs by looking at the morphological variations of the genital plate and wing venation patterns.

1. Materials and Methods

Collection and preparation of samples. Samples were collected from a rice field in Tacurong, Sultan Kudarat, Philippines (see Fig. 1). Collecting was done manually by picking up the RBB on the rice plant placing it in a container with ethyl alcohol. A total of 89 specimens were collected consisting of 44 males and 45 females. The specimens were brought in the laboratory and the sexes were identified based on the appearance of the genital plate. Males have a saddle-shaped abdominal tip while females have characteristic triangular warts (see Fig. 2). The outer wings were removed and placed in 1“x3” clear glass slides. Digital images of wings were taken using a 7.2 mega pixel Sony cyber-shot camera with 640x480 in dimension. Wing vein patterns were compared and grouped based in similarity. Adobe Photoshop was used to trace the venation patterns of the wings.

![Map of Mindanao, Philippines](https://www.eastwestcenter.org)

**Fig. 1** Geographical presentation of the ampling site in Tacurong, Sultan Kudarat, Philippines. Source: [www.eastwestcenter.org](http://www.eastwestcenter.org).

![Male and Female Genital Plates](https://example.com/male_female_genital_plates.jpg)

**Fig. 2** Male and female genital plates showing a saddle-shaped abdominal tip for male and triangular warts for female.

![Sample of the outer wing of the rice black bug, *Scotinophara* sp.](https://example.com/sample_outer_wing.jpg)

**Fig. 3** Sample of the outer wing of the rice black bug, *Scotinophara* sp.
**Landmark assignments.** Two-dimensional Cartesian coordinates of 16 landmarks for male and 17 landmarks for female were digitized by tpsDig ver.2 software (Fig.4) (Rohlf, 2004). All specimens were digitized with three replicates in order to reduce the measurement of error (Dvorak *et al.*, 2005). With this software, x and y coordinates of the landmark points which are the raw data used for further analysis were obtained. The obtained landmark configurations were then scaled, translated, and rotated against the consensus configuration by General Least Squares (GLS) Procrustes superimposition method (Bookstein, 1991; Rohlf and Marcus, 1993; Dryden and Mardia, 1998).

**Fig. 4** Designated landmarks and pseudolandmarks of the male and female genitalia.

Relative warps analysis. The technique of Relative Warp analysis corresponds to a Principal Component analysis (PCA) of the covariance matrix of the partial warp scores (Frieß, 2003). As with PCA, the most informative are the first and the second relative warps (Hammer *et al.*, 2001). In the present study, relative warp analysis was performed using the tpsRelw ver. 1.46 (Rohlf, 2008) following the algorithms developed by Bookstein (1991).

Thin-Plate Splines. In order to graphically illustrate patterns of shape variations based on the landmarks which represents the transformation of the reference to each specimen, Bookstein (1991) used the Thinplate splines. The principal warps were calculated from the reference configuration to define a set of coordinate axes for tangent space approximating the curved shaped space to which the shapes of specimens can be compared using standard linear statistical methods. The resulting x- and y- coordinates of the aligned specimens onto the principal warp axes are then projected.

**2. Results And Discussion**

Forewing patterns exceed the total number of RBB’s examined. As an example, a total of 89 wing types were observed among the 45 females examined. On the other hand, 88 wing types were documented among 44 males observed. A total of 177 wing types were documented and shown in figure 5. Results showed that all the rice black bugs exhibit widely differing wing venation patterns. The most important variations are in the type and number of marginal cells on the anterior distal part of the wing. In some forms, the marginal cells are closed and in others, the cells are open. The marginal cells are aligned in two rows in a few numbers of forms, while they are present in just a single row in most forms. The marginal cells vary in number from 1-6 while in some cases they are absent.

The longitudinal veins are also characteristically variable in terms of number and pattern. Some longitudinal veins are forked while others run straight towards the distal margin of the wings. In some forms, two longitudinal veins meet and form an enclosed cell. There are some where the longitudinal veins are continuous with the marginal cells while some form loops and netted venation systems. These results showed no sex-related differences in wing pattern observed.

The lack of a stable wing pattern for this population of RBB implies that this insect cannot independently regulate the presence or absence, the number and placement, and possibly the thickness and flexibility of the crossveins (Marcus, 2001). This capacity for the modulation of crossovein structure in many different ways may contribute to the high phenotypic plasticity in the RBB. Unlike the wings of the RBB,
individual genetic programs control differentiation and position of each of the longitudinal veins in *Drosophila melanogaster* (Weisman, 2005). Thus, the wing pattern in fruit flies appear consistent for each species.

The result of the relative warp analyses entailed that the shape of the genital plates must be controlled by polygenes based on the fact that the distribution of the shape residuals defined by the relative warps follow a normal curve. A polygenic system controlling the shapes of the genitalia has been found true for other species of organisms including the carabid beetles, *Carabus* sp (Sasabe *et al*., 2007). In addition, a Fourier analysis on two related species of Noctuid moths, *S. latifascia* and *S. descoinsi*, suggest that morphological differences may be controlled by polygenic system (Monti *et al*., 2008). This result is suggestive of a high amount of plasticity in the shape of the genitalia, which is supposedly as stable trait. This has implications to the systematics and classification of the RBB.

Little is known about the biology and ecology of the species of RBB in the Philippines. The diversity inherent in populations of RBB was the basis for classifying them into twenty four species taxa by Barrion *et al*. (2007). Among the characters used in describing the species are wing form and venation and genital plates. However, the results of this study showed that a high degree of intrapopulational variation in wing venational characteristics and genital plates of RBB from Tacurong, Sultan Kudarat.

Results of the study strongly showed the existence of morphologic diversity in rice bugs. Phenotypic plasticity might account for the inter population variation of rice black bug.
TABLE 1
VARIABILITY IN GENITAL SHAPES AMONG THE RICE BLACK BUGS AS EXPLAINED BY THE SIGNIFICANT RELATIVE WARP.

<table>
<thead>
<tr>
<th>RW</th>
<th>MALE</th>
<th>FEMALE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-Variation in the length and width aspect ratio</td>
<td>-Variation in the lateral and postero-lateral margin of the genital plate</td>
</tr>
<tr>
<td></td>
<td>-Variation in the antero-lateral margin of the genital plate</td>
<td>- Differences in the shapes of the anterior margins of the genital plates with those in RW1 having convex anterior margin and those in the +RW1 with concave anterior margin</td>
</tr>
<tr>
<td></td>
<td>- Differences in the shapes of the anterior margins of the genital plates with those in -RW1 having convex anterior margin and those in the +RW1 with concave anterior margin</td>
<td>-Describes asymmetry in the shape of the genital plate</td>
</tr>
<tr>
<td>2</td>
<td>-Variation in the antero-lateral margin of the genital plate</td>
<td>(-)RW2 concave anterior margin and (+)RW2 convex anterior margin</td>
</tr>
<tr>
<td></td>
<td>- Differences in the shapes of the anterior margins of the genital plates with those in RW2 having convex anterior margin and those in the +RW2 with concave anterior margin</td>
<td>-Describes asymmetry in the overall shape of the genital plate</td>
</tr>
<tr>
<td>3</td>
<td>-Variation in the antero-lateral and postero-lateral margin of the genital plate</td>
<td>- Variation in the length-width aspect ratio</td>
</tr>
<tr>
<td></td>
<td>- Differences in the shapes of the anterior margins of the genital plates with those in RW3 having concave anterior margin and those in +RW with convex anterior margin</td>
<td>- Separates symmetrically shaped genitalia from the asymmetrical ones.</td>
</tr>
<tr>
<td></td>
<td>-Describes asymmetry in the shape of the genital plate</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>- Differences in the shapes of the anterior margins of the genital plates with those in -RW4 having concave anterior margin and those in +RW4 with convex anterior margin</td>
<td>-Describes asymmetry in the shape of the genital plate</td>
</tr>
<tr>
<td></td>
<td>- Describes asymmetry in the shape of the genital plate</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>- Differences in the shapes of the anterior margins of the genital plates with those in -RW5 having concave anterior margin and those in +RW5 convex anterior margin</td>
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Phenotypic plasticity is a common characteristic among insects which has the ability of its genotype to exhibit variable phenotypes in such variable environment (Pfenning et al., 2010). Phenotypic plasticity can be adaptive (improving organism’s survival and reproduction) or sometimes may not be adaptive, due to its biochemical, physiological and developmental biology which makes them plastic (Pigliucci et al., 2006). A low rate of phenotypic plasticity yields a low rate of adaptability of insect which may provide opportunities in control and management (Sakai et al., 2001, Stockwell et al., 2003). Thus, the more plastic an organism is the more adaptive they are to environmental conditions.

Moreover Rice black bugs vary in terms of population size and maybe characterized as either non-outbreak or outbreak populations based on density. Sometimes, differences in the phenotypes can be observed between non-outbreak and outbreak populations of pests. Such is the case of the migratory locust Locusta migratoria where population density significantly influences its morphology.
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(Chapuis et al., 2008). This form of phenotypic plasticity is termed as density dependent phase polyphenism. Technically, this phenomenon occurs when different rearing densities during juvenile stages produce individuals that vary in a variety of traits including morphology (Jannot et al., 2009).

Results showed no differences in the pattern of variability in wing venation systems and genital plates among individuals within the population, an implication that RBB may not exhibit density dependent phase polyphenism. However, tests should be conducted to determine the plausibility of genetic structuring among RBB populations based on the propensity to outbreak (Chapuis et al., 2008). This is to test whether outbreak events in RBB are strictly not determined by solely environmental factors, but also in part by the expression of traits that are adaptive and under genetic control.

CONCLUSION

Results of this study showed extremely high inter-individual variation in wing venation patterns. The wings were found to differ in the number and arrangement of the marginal cells and the longitudinal veins. Comparison of the shapes of both the male and female genital plates using the method of geometric morphometrics revealed continuous variation in the concavity of the anterior margins, and disparity in the length-width aspect ratio of the structures. This variability might be indicative of a high amount of genetic diversity for this population of RBB, which has important implications to the management of this pest.

Further studies should be conducted to determine the genetic basis for the observed diversity in forewing venation patterns and shape of the male and female genitalia. Test in determining plausibility of genetic structuring among RBB populations based on the propensity to outbreak should also be conducted to determine whether outbreak events of RBB is solely dependent on environmental factors or does it also manifest adaptive expression of traits or under genetic control.

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Fig. 6 Summary of the geometric morphometric analysis showing the consensus morphology (uppermost panel) and the variation in the shapes of the genital plate among male and female populations of RBB in Tacurong, Sultan Kudarat.
REFERENCES


