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Describing the Shapes of the Mandibles of the Rice Leaf Folder Larvae, *Marasmia patnalis* Bradley Feeding on Different Rice Varieties using Elliptic Fourier Analysis

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ABSTRACT

Elliptic Fourier (EF) analysis was used to describe mandible shape variation in the rice leaf folder, *Marasmia patnalis,* feeding on different rice varieties. Canonical variate analysis (CVA), multivariate analysis of variance (MANOVA) and principal component analysis was used to select the Fourier coefficients with the best discriminatory power. The results of the analysis show variations in the shape of the left and right mandibles. Considerable differences in mandible shapes within and between populations of the pest feeding on different rice variety based on the results of CVA and MANOVA analysis of shape variables. The results suggest that the resistance factors inherent to the rice varieties may have played an important role in the differentiation of the populations of the pest.

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INTRODUCTION

Marasmia patnalis is one species of leaffolders that is considered a major pest of rice in many parts of rice-growing countries. Host-plant resistance has played a very important role in the management of this pest but when deployed in the field, many succumb to the attacks of the pest. Many of the modern rice varieties that are resistant to the pest have become vulnerable to infestation leading to outbreaks (Demayo *et al*., 2011). The ability of this species to survive on rice hosts with specific genes for resistance is a useful strategy and adaptive advantage for their better survival in the ecosystem (Khiaban *et al*., 2010). It is hypothesized that when this species has two or more rice host variety, the possibility arises that gene flow is restricted and are subjected to divergent natural selection for host adaptations (Berlocher and Feder, 2002). With the host plant species producing different selective regimes, genetic variations and host plant associated local adaptation may occur (Ruiz-Montoya *et al.*, 2003). These variations maybe reflected in the phenotype (Novotny and Basset, 2005) especially on the feeding mouthparts such as the mandible. The strong mandible of the larva allow regular scraping on the growing paddy leaves longitudinally resulting in papery dry leaves (Chatterjee, 1979). Since the larvae are the ones causing severe infestations and annihilate the rice plant totally, it is hypothesized that the shapes of their mandibles vary depending on the rice variety. It is for this reason that we used advanced methods in computational biology integrating statistics, computer imaging and geometry to describe the variations observable in the mandibles of those larvae utilizing or feeding on rice types with differences in resistance factors. We used a method based on Elliptic Fourier descriptors (Kuhl and Giardina, 1982) for the analysis of the left and right mandibles of *M. patnalis* feeding on different rice varieties. This method describes an overall shape mathematically by transforming coordinate information concerning its contours into Fourier coefficients. Principal component analysis for summarizing the elliptic Fourier descriptors was suggested by Rohlf and Archie (1984) and has been used for several studies, such as begonia leaves (McLellan, 1993), soybean leaflets (Furuta *et al*., 1995), buckwheat kernels (Ohsawa *et al*., 1998), yam tubers (Toyohara *et al*., 2000), radish roots (Iwata *et al*., 1998) and citrus leaves (Iwata *et al*., 2002). Recently, Iwata and Ukai (2002) developed a program package (SHAPE) for the quantitative evaluation of biological shapes on the basis of elliptic Fourier descriptors, thus, was used in this study.

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MATERIALS AND METHODS

Collection and Identification of the Larvae"

Opportunistic sampling of rice leaf folder larvae was done from varieties with specific genes for resistance from rice farms in Manticao, Misamis Oriental, Philippines (Figure 1). Rice leaves which have white mark feeding strips indicate the presence of a larva. Each collected larva was identified using a dissecting microscope.

Fig. 1: Topographic view showing the sampling site in Misamis Oriental (Manticao). Source: www.map.google.com

Table 1: List of host rice varieties and their corresponding characteristics. Source: www.irri.org

Rice Variety	Ave. Yield (t/ha)	Growth Duration (days)	Height (cm)	Susceptibility
NSIC Rc160	5.6	122	96	MS & R
IRBB2 (V10)	-	-	-	MS

Legend: MR- Moderately Resistant; R – Resistant; I – Intermediate; MS – Moderately Susceptible; S – Susceptible

Processing of The Mandible:

Under the stereomicroscope, the mandibles (Fig. 2) were separated from the body using a dissecting needle and mounted on clear glass slides. Glycerol was used to avoid accumulation of bubbles in the slides. The image of the mandibles was captured using a Canon Kiss X4 DSLR.

Elliptic Fourier Analysis:

For outline analysis of the mandibles of the rice leaf folder, the software package SHAPE v.1.3 was used (Iwata and Ukai, 2002). It is based on the methodology of Elliptic Fourier descriptors which allows describing each type of two-dimensional shape with a closed outline, in terms of harmonics. All images were saved in .bmp format (24bit) and was binarized with Chain Coder before tracing the outlines in Chain-code, a coding system that describes the geometrical information on the shapes. Then, the Chain-code file was transformed into a Normalized Elliptic Fourier file with Chc2Nef, using 20 harmonics. It allows detailed analysis of fine-scale morphological variation in the outline of the mandibles of rice leaf folder's larvae. The matrix of the harmonic coefficients underwent normalization based on the first harmonic, the data transformed into shape variables. Subsequently, a PCA was performed on the variance-covariance matrix of normalized coefficients (elliptic Fourier descriptors) using PrinComp, which gives a graphical output of the average shape± the standard deviation (Magrini and Scoppola, 2010). Principal component scores were further subjected to Kruskal-Wallis test, a non-parametric version of one way ANOVA, to determine if the populations differ significantly from one another based on the shape of its mandible. Box and whiskers plot was used to visualize the distribution of different rice leaf folder populations. Multivariate and statistical analysis were done using the software PAST version 1.91 as platform (Hammer *et al.*, 2001) (Fig. 3).

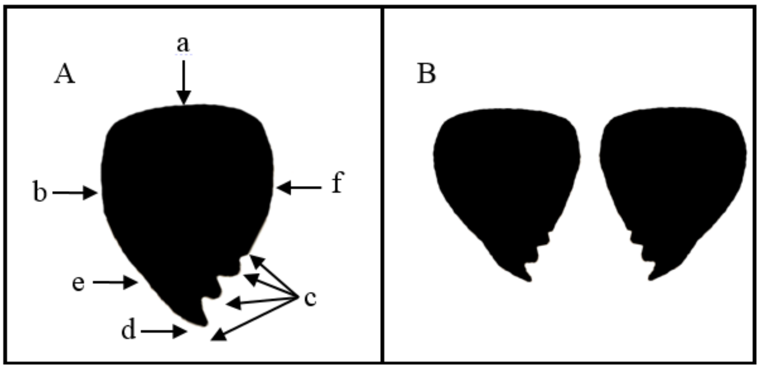

Fig. 2: (A) Representation of the mandible showing its different parts (a = mandible attachment site; b = external margin; c = incisor teeth; d = basal angle; e = basal margin; f = internal margin) and (B) *M. patnalis* mandibles.

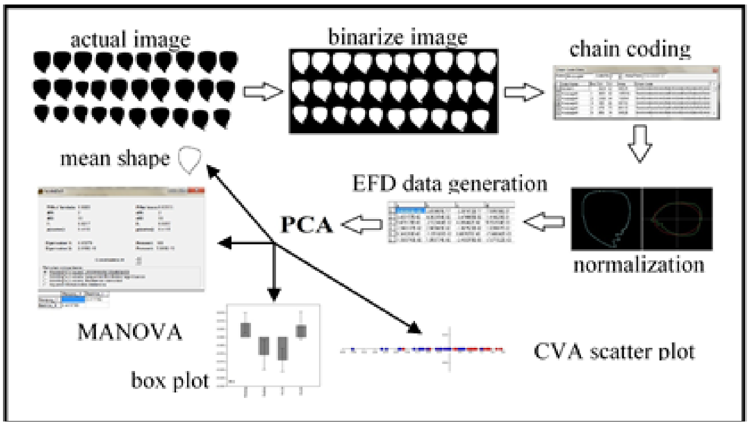

Fig. 3: Outline of the Elliptic Fourier Analysis of the rice leaf folder's larvae mandible shape.

RESULTS AND DISCUSSIONS

The variations in shapes of the mandibles of *M. patnalis* as shown by the results of the canonical variate analysis and multiple analysis of variance of shape variables are presented in Figure 4 and Table 2. While variations are evident, it can be seen from the results that differences between the 2 populations of the pest was much defined in the left mandible. Variations in shapes was evaluated based on the results of the principal component analysis shown in Table 3. The 3 significant principal component scores analyzed using Kruskal-Wallis test (Table 4) showed the nature of the differences as shown by the generated boxplots (Fig. 5). The visualization was based on the coefficients of the elliptic Fourier descriptors recalculated inversely using an eigen-vector matrix, letting the score on a particular principal component be equal to the standard deviation, while the scores on the remaining components remained at the mean. It can be seen from the results that *M. patnalis* that survived in the 2 short leaved, medium duration and non-aromatic rice varieties has semi-rounded mandible attachment site, pointed basal angle and with the less number of teeth that are less prominent and extensive as described by PC2 and PC3 (Tables 3&4).

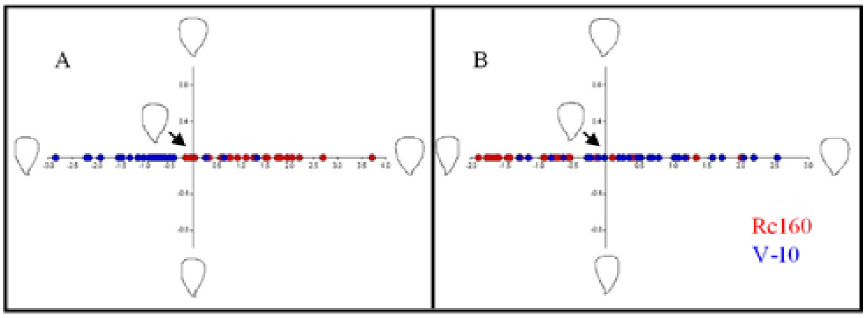

Fig. 4: Scatter plot showing the distribution of the (A) left and (B) right mandible shapes of *M. patnalis* feeding on RC160 and V-10 rice varieties

Table 2: Results of MANOVA for significant variation in the shape of the left and right mandible of *M. patnalis.*

	Wilk's Lambda	df1	df2	F	P
left	0.5387	7	50	6.116	3.464E-05
right	0.7685	8	49	1.846	0.09091

Table 3: Percentage of variance and overall shape variation in the left and right mandible of *M. patnalis* as explained by each of the significant principal component.

PC	Left	PC	Right
1 53.1857%	Variation in the interior margin, external margin, basal margin, basal angle, protrusion,position, number and length of the teeth.	1 50.2463%	Variation in the external margin, basal margin, basal angle, protrusion,position, number and length of the teeth.
2 17.8684%	Variation in the site attachment of the mandible to head, basal angle, protrusion, position, number and length of the teeth.	2 23.8203%	Variation in the site attachment of the mandible to head, external margin, interior margin, basal margin, basal angle, protrusion,position, number and length of the teeth.
3 9.0091%	Variation in the basal angle, protrusion, position, number and length of the teeth.	3 8.1483%	Variation in the interior margin, basal margin, basal angle, protrusion,position, number and length of the teeth.

Table 4: Results of Kruskal-Wallis test for significant differences in the left and right mandible shapes of *M. patnalis* feeding on RC160 and V-10 rice varieties.

	Left				Right		
		Rc160	V-10			Rc160	V-10
PC1				PC1			
	Rc160	-	0.4554		Rc160	-	0.8764
	V-10	0.4554	-		V-10	0.8764	-
PC2				PC2			
	Rc160	-	8.074E-06		Rc160	-	0.6242
	V-10	8.074E-06	-		V-10	0.6242	-
PC3				PC3			
	Rc160	-	0.0843		Rc160	-	0.001357
	V-10	0.0843	-		V-10	0.001357	-

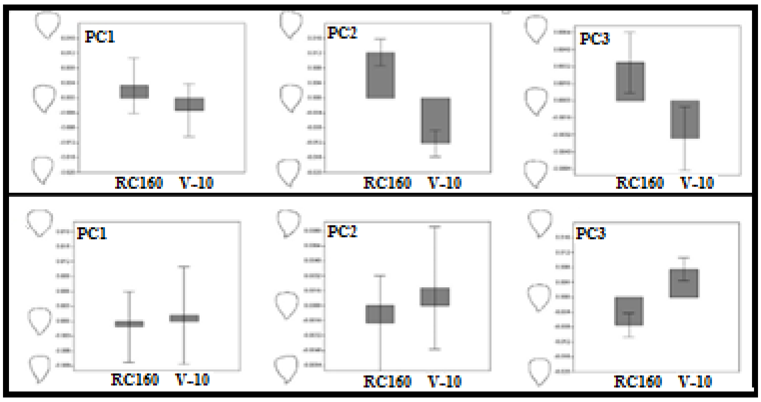

Fig. 5: Box and whiskers plot of the significant principal component in the (A) left and (B) right mandible shapes of *M. patnalis* feeding on Rc**160** and **V-10**).

The results of this study showing variations between populations of *M. patnalis* feeding on two rice varieties with different genes for resistance could be due to a number of factors or mechanisms that may be involved in the evolution of host plant selection behavior in the pest such as the quality of the two rice varieties which may have differed for larval development with adult females prefer to oviposit specifically on varieties where offspring fitness will be higher. Many studies have shown that preference may have been favoured for a variety of reasons such as variation in host quality or abundance (Rausher, 1978, 1980; Papaj, 1986a; Dukas & Ellner, 1993; Dukas & Clark, 1995). Likewise nutritional value, microhabitat or the abundance of natural enemies may have contributed also to the differences as shown in selected studies (Jaenike, 1978; Wiklund, 1981; Singer, 1983; Ward, 1987; Courtney *et al.*, 1989). Other studies also suggest that specificity in the interactions between the pest and the rice varieties is an important factor that are dictated by the pest's ability to disperse among multiple host species. Those that differ in their ability to disperse among multiple hosts are expected to differ in their degree of host specificity and ultimately in the degree of co-speciation observed between them (Clayton *et al.*, 2002). This means that those performance traits, and their association to morphology such as the mandible shape and ecology, are of cornerstone importance for determining the role and relative contribution of the aforementioned processes in shaping the morphological variation observed in this study (Kaliontzopoulou *et al*., 2011).

Conclusion:

This study has shown that quantitative analysis of the mandible shape of *M. patnalis* using principal component scores obtained from standardized elliptic Fourier descriptors can provide clear understanding of the variations in structures not easily done using conventional morphometric and qualitative analysis. Since the principal components are independent of each other, visual observations how each principal component affects the shape by drawing the contours under particular score value conditions, principal component scores can be used as new shape characteristics of *M. patnalis* feeding on different host rice varieties. The use of elliptic Fourier descriptors and principal component analysis showed two major advantages in the study. First, this method accurately detected the small shape variations. The MANOVA based on the component scores clearly depicted the significant variations among different host rice varieties. Secondly, it evaluated the shapes of mandible independently.

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