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Elliptic Fourier analysis of Mandibular Shapes of the Rice Leaf Folder *C. Medinalis* Guenée Feeding on Different Rice Varieties

Christine Lovelle A. Mahinay[1] and Cesar G. Demayo[2]

[1]*Xavier University, Cagayan de Oro City*
[2]*Department of Biological Sciences, MSU-Iligan Institute of Technology, 9200 Iligan City, PHILIPPINES*

ABSTRACT

Astrong mandible of the leaf folder Cnaphalocrocismedinalislarva is used in scraping leaf tissues of rice during feeding.It is for this reason that in the control of this pest, host plant resistance traits were focused the physical and biochemical attributes of the leaf that discourages the insect from feeding. Breeding rice resistant to leaffolders have resulted to the identification of some varieties resistant to the insect pest. However, the deployment of varieties with specific genes for resistance were soon found to succumb to pest attacks. Resistance breakdown was attributed to the insect's capability to overcome the resistance factors. It is believed that certain genotypes of the insect pest were able to feed on these resistant plants and this could be due to differences in the mandibles of the larvae. This study was therefore conducted to find out whether the mandible shape of the rice leaf folder C.medinaliswould likely differ with respect to their utilization of the different rice varieties. Geometric morphometric (GM) methods particularly elliptic Fourier analysis (EFA) was employed to describe the shapes of the mandibles of C.medinalisinfesting rice varieties with different genes for resistance. Resultsof the study showed significant differences in shapes of the mandibles among populations of C.medinalisfeeding on different rice varieties. Variations in the mandible shapes could possibly be due to selection and/or co-speciation of the insect pest with the host plant.

Keywords: *C. medinalis(Guenée)*, Elliptic Fourier Analysis, host specificity, intraspecific variation

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INTRODUCTION

The rice leaf folder *Cnaphalocrocismedinalis* attack the vegetative stage of rice and is considered as a polyphagous defoliator[1]. The larva folds and scrapes the green tissues of the leaves from within, scorching and drying and eventually damagingthe rice plant resulting to great losses in terms of yield. Pesticide application was the main control measure used against the insect pest but because of the health and environmental damage caused by unabated applications, other measures were explored. Host plant resistance is an example of the alternative pest control measure against herbivorous pests [2]. While most of the modern grown crops have different level of resistance against the pest, many did not escapethe ability of the insect pest to overcome the resistance factors developed. Deployment of the varieties in different geographical regions shows differences in the level of resistance of the plant and many of which succumb to the infestation of the larvae of the insect pest. It is argued that the utilization of the rice types with different genes for resistance is considered an adaptive advantage to the pest for their survival [3]. The possibility arises that gene flow is restricted and are subjected to divergent natural selection for host adaptations [4]. It is also hypothesized that with the host plant species producing different selective regimes, genetic variations and host plant associated local adaptation in the insect may occur [5] which could reflected in the phenotype [6] (Novotny and Basset, 2005) and in the case of leaffolders, is the mandible. The mandibles, one on each side of the head, are typically the largest mouthpart of chewing insects, being used to masticate food items [7].This is used by the insect to scrape the growing paddy leaves longitudinally resulting in papery dry leaves [8]. This structure is central to understanding of adaptive modifications in insects thus is hypothesized that the shapes of the mandibles vary depending on the rice variety being utilized. This current study quantitatively describe the shapes of the mandible and find out if variations exist to those populations of the larvae that fed and survived in rice types with varying resistance factors. The methods of geometric morphometrics(GM) integrate statistics, computer imaging and geometry to quantitatively describe the variations observable in the mandibles of the larvae.Elliptic Fourier (EF) descriptors [9] was used for the analysis of the left and right mandibles by describing an overall shape mathematically by transforming coordinate information concerning its contours into Fourier coefficients. Principal component analysis was used in summarizing the elliptic Fourier descriptors [10]. The method was successful in its use in describing quantitatively the shapes of begonia leaves [11], soybean leaflets [12], buckwheat kernels[13] , yam tubers[14] , radish roots [15] and citrus leaves [16] thus was used in the current study. It is argued that understanding mandibular shape morphology of *C. medinalis*would give an idea regarding theiradaptations which could greatly contribute to their proper management.

MATERIALS AND METHODS

Opportunistic sampling of rice leaf folder larvae was done from rice farms in Bukidnon, Misamis Oriental and Lanao del Norte, 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. Larva of *C. medinalis*has two pairs of curve lines located at the lower part of the head.

Figure 1. Topographic view showing the sampling site in Bukidnon (Valencia), Misamis Oriental (Manticao) and Lanao del Norte (Kapatagan).
Source: www.google.com

Under the stereomicroscope, the mandibles 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.

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 Rc 226	6.2	112	-	MR
NSIC Rc128	5.5	118	99	R, MR & S
NSIC Rc160	5.6	122	96	MS & R
IRBB2 (V10)	-	-	-	MS
Masipag	3.8	-	-	R
Red Rice	5	155	128	R

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

For outline analysis of the mandibles, the software package SHAPE v.1.3 was used [17]. 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 [18]. 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 [19] (Hammer *et al.*, 2001).

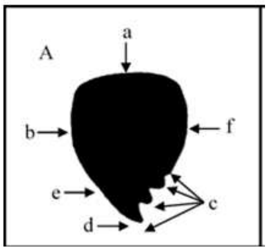

Figure 2. Representation of the mandible showing its different parts
(a = mandible attachment site; b = external margin; c = incisor teeth; d = basal angle; e = basal margin; f = internal margin).

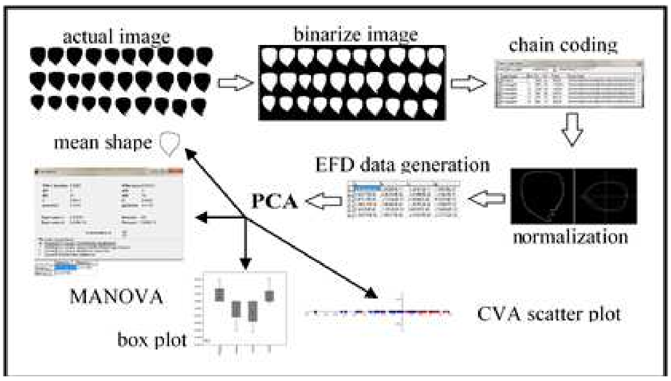

Figure 3. Outline of the Elliptic Fourier Analysis of the rice leaf folder's larvae mandible shape.

RESULTS AND DISCUSSION

The extractedFourier descriptors resulting from Elliptic Fourier Analysis were subjected to principal component analysis (PCA), an exploratory procedure to compare the mean shapes and elucidate the underlying relationships. The description of the shapes based on significant PC's are shown in Table 2. Shape diversity between populations of the pest based on the left and right mandibles were analyzed using canonical variate analysis (CVA) and multiple analysis of variance (MANOVA) (Fig. 4, Table 3). Analysis on the population distribution was done based on the overall variation in the shape of mandible. The principal component scores were further analyzed using the Kruskal-Wallis test. It was used for comparing the samples whether they are independent or not related. The results of Kruskal-Wallis test done in each of the significant principal components were shown in Table 4. The variations in the outlines of the shapes and box plots representation of the left and right mandibles feeding on different rice varieties were reconstructed as shown in Fig. 5.

Table 2.Percentage of variance and overall shape variation in the left and right mandibles of *C. medinalis* as explained by each of the significant principal component.

PC	Left	PC	Right
PC1 47.9102%	Variation in the protrusion , position , number and length of teeth.	PC1 52.0222	Variation in the basal margin, basal angle, protrusion , position , number and length of teeth.
PC2 23.3768%	Variation in the external margin, protrusion , position, number and length of teeth.	PC2 19.5765%	Variation in the basal margin, basal angle, protrusion , position , number and length of teeth.
PC3 7.6167%	Variation in the protrusion, position and length of teeth.	PC3 6.6652%	Variation in the interior margin, basal angle, protrusion , position and length of teeth.
PC4 5.1381%	Variation in the site attachment of mandible to the head.	PC4 5.7394%	Variation in the site attachment of mandible to the head, interior margin, basal angle, protrusion, position, number and length of teeth.
PC5 2.8074%	Variation in the protrusion, position, number and length of teeth.	PC5 2.7830%	Variation in the site attachment of mandible to the head, interior margin, basal angle, protrusion, position, number and length of teeth.
PC6 2.3142%	Variation in the basal angle, interior margin, protrusion, position, number and length of teeth.	PC6 2.6909%	Variation in the basal angle, protrusion, position, number and length of teeth.
PC7 1.8213%	Variation in the protrusion, position and length of teeth.	PC7 1.9402%	Variation in the basal angle, protrusion, position, number and length of teeth.
PC8 1.4195%	Variation in the protrusion, position and length of teeth.	PC8 1.4119%	Variation in the basal angle, protrusion, position, number and length of teeth.

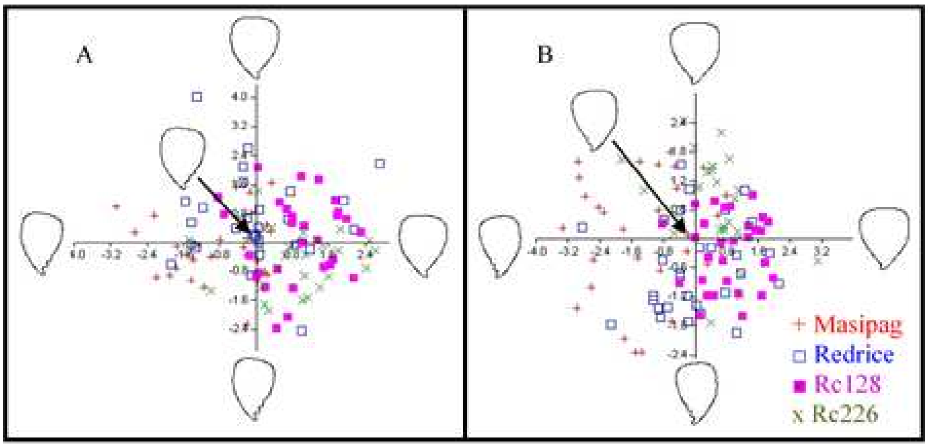

Figure 4 CVA scatter plot showing the distribution of *C. medinalis* obtained from Kapatagan and Valencia populations based on the (A) left and (B) right mandible shape and rice varieties they infest.

Table 3.Results of MANOVA for significant variation in the shape of the left and right mandible of *C. medinalis*

		Wilk's Lambda	df1	df2	F	p(same)
Masipag (R) and Redrice (R)	left	0.7821	8	78	2.716	**0.01079**
	right	0.6645	8	49	3.093	**0.006649**
Rc128 (R,MR&S) and Rc226 (MR)	left	0.8794	8	49	0.8399	0.5724
	right	0.7112	8	49	2.487	**0.02382**
Between varieties	left	0.5571	24	389.2	3.625	**4.74E-08**
	right	0.4348	24	305.1	4.229	**1.113E-09**

Table 4. Results of Kruskal-Wallis test for the significant differences in the left and right mandible shapes of *C. medinalis*feeding on different rice varieties.

	Left						Right				
		Masipag	Redrice	Rc128	Rc226			Masipag	Redrice	Rc128	Rc226
PC1	Masipag	0	0.06044	**0.01214**	0.8606	PC1	Masipag	0	0.1237	**0.009837**	0.7032
	Redrice	0.3626	0	0.744	0.1941		Redrice	0.7419	0	0.401	0.1396
	Rc128	0.07286	1	0	**0.0227**		Rc128	0.05902	1	0	**0.007137**
	Rc226	1	1	0.1362	0		Rc226	1	0.8375	**0.04282**	0
PC2	Masipag	0	0.7492	0.05122	0.2026	PC2	Masipag	0	0.8764	0.253	0.3671
	Redrice	1	0	0.1836	0.2342		Redrice	1	0	0.164	0.3158
	Rc128	0.3073	1	0	0.3924		Rc128	1	0.9837	0	0.6464
	Rc226	1	1	1	0		Rc226	1	1	1	0
PC3	Masipag	0	0.3375	**0.00192**	**0.0002034**	PC3	Masipag	0	0.1687	**0.04321**	0.6187
	Redrice	1	0	**0.03312**	**0.008389**		Redrice	1	0	**0.003208**	0.1275
	Rc128	**0.01152**	0.1987	0	0.9071		Rc128	0.2593	**0.01925**	0	**0.04484**
	Rc226	**0.00122**	0.05034	1	0		Rc226	1	0.765	0.2691	0
PC4	Masipag	0	0.06423	**0.01722**	**0.002918**	PC4	Masipag	0	**0.008012**	**0.0002281**	0.09153
	Redrice	0.3854	0	0.5756	0.2192		Redrice	**0.04807**	0	0.6133	0.2078
	Rc128	0.1033	1	0	0.539		Rc128	**0.001368**	1	0	**0.01229**
	Rc226	0.01751	1	1	0		Rc226	0.5492	1	0.07373	0
PC5	Masipag	0	0.5553	0.114	0.7424	PC5	Masipag	0	0.2163	0.2832	0.5442
	Redrice	1	0	0.3838	0.7975		Redrice	1	0	0.7915	0.06202
	Rc128	0.684	1	0	0.1314		Rc128	1	1	0	**0.01734**
	Rc226	1	1	0.7886	0		Rc226	1	0.3721	0.104	0
PC6	Masipag	0	0.8114	0.5314	0.4966	PC6	Masipag	0	0.1199	**0.002975**	**0.0001728**
	Redrice	1	0	0.5339	0.4554		Redrice	0.7195	0	0.2222	**0.01627**
	Rc128	1	1	0	0.938		Rc128	**0.01785**	1	0	0.07123
	Rc226	1	1	1	0		Rc226	**0.001037**	0.09762	0.4274	0
PC7	Masipag	0	**0.003899**	0.1932	0.7424	PC7	Masipag	0	0.8764	0.07371	0.1761
	Redrice	**0.0234**	0	0.2078	**0.01125**		Redrice	1	0	0.05988	0.1761
	Rc128	1	1	0	0.2974		Rc128	0.4423	0.3593	0	0.5546
	Rc226	1	0.06747	1	0		Rc226	1	1	1	0
PC8	Masipag	0	0.6109	**0.01041**	**0.03033**	PC8	Masipag	0	0.3084	0.09006	**0.00213**
	Redrice	1	0	0.09305	0.2078		Redrice	1	0	0.597	0.07123
	Rc128	0.06245	0.5583	0	0.6078		Rc128	0.5403	1	0	0.1481
	Rc226	0.182	1	1	0		Rc226	**0.01278**	0.4274	0.8886	0

Studies have shown morphological, anatomical, and physiological and biochemical factors each controlled by different sets of genes are associated with resistance in plants [20]. These enable them to avoid, tolerate or recover from the effects of pest attacks and even considered proof to be a successful tool against pests [21]. However, thereare also the existence of variant forms in pests which possess the capability to overcome the nature of the resistance factors in the rice variety. Results of this study for example have shown significant differences in mandible shapes in *C. medinalis* attacking rice with different level of resistance. Differences in mandible morphology and bite force in individuals in this species can be considered a relevant feeding strategy in the pest[22]. The ricevarieties with different resistance genes may have played a great role in mandible shape variation and bite performance diversification [23]-[25]. This can be supported by studies that have shown *C. medinalis*vary in preference to different rice varieties [26]. The different rice varieties having different sets of resistance genes used in the studyserved as selection regimes to the pest [5], 27-29] allowing those possessing stronger mandibles with larger and more define teeth to successfully feed on its plant host. We can therefore conclude that the relationship between *C. medinalis* and its rice plant host is a dynamic one which may favor either the pest or the plant by either discouraging the pest's attention by various defense strategies or it may encourage visitations by those having different adaptive mechanisms to exploit their host plants [30]. Rice host-plant diversity therefore also promote high taxonomic diversity and ecological disparity among the insect herbivore *C. medinalis*[31].

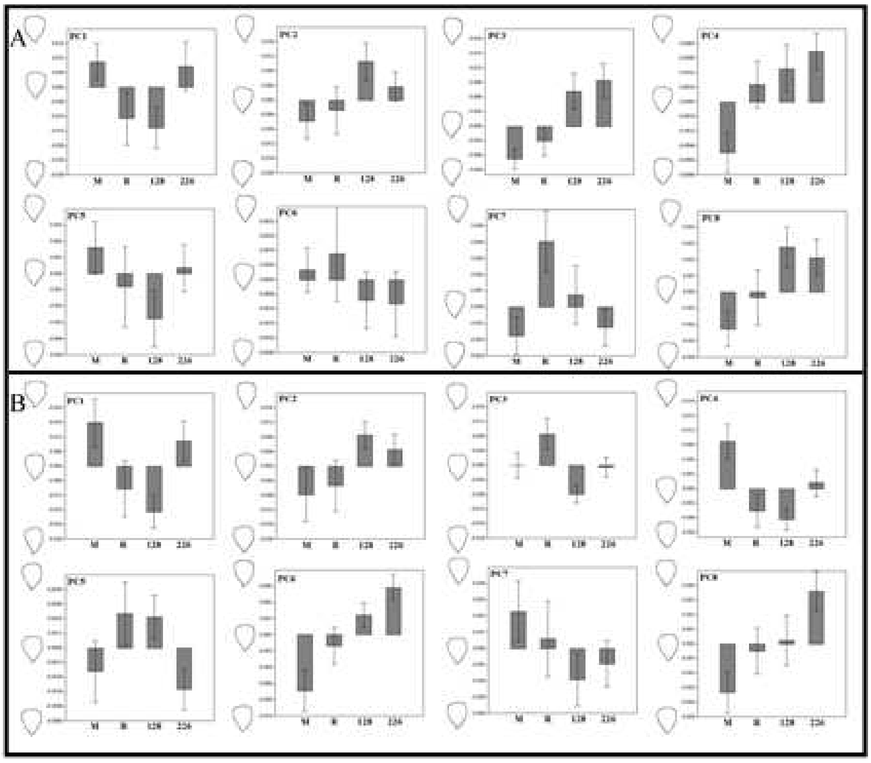

Figure 5 Box and whiskers plot of the significant principal component in the (A) left and (B) right mandibles of *C. medinalis*feeding on Masipag (M), Redrice (R), Rc128 and Rc226 rice varieties.

CONCLUSION

Differences in the mandible shapes of rice leaf folder species, *C. medinalis,* feeding on different rice varieties were shown to differ based on elliptic Fourier analysis. Principal component analysis of Fourier descriptors showed that the variation was mainly due to the difference in the basal angle, basal and external margin and different aspects of the mandible. It is argued that the rice host-plants promote high taxonomic diversity and ecological disparity in*C. medinalis* as shown by the variations observed in their mandibular shapes.

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