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Landmark and Outline Methods in Describing Petal, Sepal and Labellum Shapes of the Flower of Mokara Orchid Varieties

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ABSTRACT

Together with petal color and other floral characteristics, petal shape and size vary widely within Mokara orchids. An appropriate understanding of their flower forms is fundamental and necessary for the improvement of this economically and aesthetically important plant. This study was therefore conducted to describe variations in the flowers of Mokara with emphasis on the shapes of sepals, petals and labellum using geometric morphometric (GM) methods based on summarized information about contours of the structures. We specifically applied the truss-network based analysis of landmarks and the elliptic Fourier method in describing the petal and sepal outlines. Each of the methods required a dimension reduction technique in the form of principal component analysis to summarize independent shape characteristics. Results showed that classification and discrimination of the varieties based on the shapes of the petals and sepals could be achieved using both techniques. © 2011 Friends Science Publishers

Key Words: Geometric morphometrics; Landmarks; Truss-network; Elliptic fourier

INTRODUCTION

Mokara (*Mokara spp.*), commonly named the "Smile Orchid", is native to Asia, where it has been first discovered and cultivated. It is a Vandaceous orchid resulting from a trigeneric hybridization between the Ascocentrum, Vanda and Arachnis Orchids. The first Mokara hybrid was created in Singapore in 1969 and was called Mokara Wai Liang, named after C.Y. Mok. Since then, several varieties were produced, where many of the hybrids have unique and highly variable star-shaped flowers. This orchid has flowers with the largest number of colors compared to other orchids like purple, pink, blue, red, orange, yellow, and coral with each and every color has its own range. The flower consists of three sepals, which are usually different from the petals in shape but not in color. There are also three petals but one has been greatly modified into a lip or labellum, which is an extremely complex structure and quite different from the other two sepals (Soon, 1989). This orchid group is therefore interesting to study quantitatively by describing the shapes of the sepals, petals and labellum of the flower. Since flower shape is one of the most important characteristics among indices used in variety registration, there is need for a more acceptable mechanism of evaluation.

Diversity of organisms is normally based on described based on morphological forms (Adams *et al.*, 2004). Qualitative evaluation is usually done and is based only qualitatively based on human visual judgment thus usually leads to unacceptable human errors. A good method of quantitative measurement of flower characteristics such as shapes is therefore needed. Early in the 20th century, biology had undergone transition from descriptive field to a quantitative science (Bookstein, 1998). At present, quantitative measures such as aspect ratio, petal area and perimeter are usually used in flower shape analysis but still these are not highly accurate. A more objective and quantitative evaluation method of petal shape has emerged since then. With the advances in digital photography, digital image analysis has been very useful in studying morphological shapes. Coupled with the development of the different statistical methods such as analysis of variance (Fisher, 1935), correlation coefficient (Pearson, 1895) and principal component analysis (Pearson, 1901; Hotelling, 1933) further advanced the quantitative rigor. By the mid of the twentieth century, the modern field of morphometrics began, where description of biological forms and the patterns of shape variations were described quantitatively with the combined concepts of statistics (Adams *et al.*, 2004). Among these methods of image analysis, the elliptic Fourier analysis (EFA) (Kuhl & Giardina, 1982) and the landmark-based methods of geometric morphometrics (GM) are commonly used. The applications of GM in shape analysis had increased dramatically as shown by the number

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of publications implying the increasing role of this method in many biological researches (Adams *et al.,* 2004).

Geometric morphometrics is technique that has generated valuable results in many fields of classic morphometry (Shipunov & Bateman, 2005). Elliptic Fourier analysis or EFA is the most popular form of Fourier shape analysis (Ferson *et al.,* 1985). This method uses a closed contour or outline of an object to calculate several shape curves and elliptic Fourier coefficients, which represent the curves can then be used as variables for multivariate analysis (Jensen *et al.,* 2002) such as principal component analysis for summarizing the elliptic Fourier descriptors. Likewise, the shape of the structure can be quantitatively analyzed by using the principal component scores as ordinary quantitative characteristics. Several studies have applied the use of elliptic Fourier descriptors to analyze the plant organ shapes like the leaf of *Betula* (White *et al.,* 1988), 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).

On the other hand, landmark-based approach uses well-defined points which are homologous from one specimen to the next (Hammer *et al.,* 2001) and involves studies on the structures that Cartesian coordinates can be taken (Bookstein, 1991; Rohlf, 1999; O'Higgins, 2000; Pavlinov, 2001; Adams *et al.,* 2004; Zelditch *et al.,* 2004). These points or so-called landmarks are placed on the image pinpointing the most important location on the object (Shipunov & Bateman, 2005). The method of relative warps and shape coordinates (Bookstein, 1991) for the analysis of morphometric variation based on landmark data gives a special help to anatomists (Lockwood *et al.,* 2002) and taxonomists (Rohlf, 1993; Alibert *et al.,* 2001; Gumiel *et al.,* 2003).

We used the truss-network based analysis of landmarks using a generalized-least square fitting algorithm and the elliptic Fourier method. Understanding the variations among sepals and petals described with the use of elliptic Fourier analysis and the variations in the lip or labellum described with the use of landmark method will advance our understanding variability in the shapes of this highly color variable orchid group.

MATERIALS AND METHODS

Four varieties of orchids belonging to genus Mokara were used in this study. These four orchid varieties were collected from JC Gardens Orchids and Green at Carpenter Hills Koronadal City, Philippines. Four Mokara varieties included are Mokara Salaya Red, Mokara Kultana Gold, Mokara Red and Mokara Sayan Gold. The 3 sepals, 2 petals and the labellum were separated and were arranged accordingly between the two (2) clear glasses. The images of the sepals, petals and labellum were captured using a scanner (Scanjet HP 2400) at 600 dpi resolution. The images were separated or grouped accordingly into right, left and center sepals, right and left petals and labellum. The sepals and petals were used to discriminate the different varieties of Mokara using the elliptic Fourier analysis while the lip or labellum were used to discriminate the different Mokara varieties using the landmark method.

The outline of sepals and petals were obtained by converting the full colored bitmap image into a binary (black & white color) images. The outline of the images were then traced and recorded as chain code (Freeman, 1974). Using the software CHC2NEF the normalized EFDs were calculated based from the chain code information (Kuhl & Giardana, 1982). Princomp software was used to visualize variations among Mokara sepals and petals by reconstructing their shapes using the elliptic Fourier coefficients by inverse Fourier transformations (Furuta *et al.,* 1998). These processes were made possible by the development of computer program package "SHAPE" (Iwata & Ukai 2002) based on elliptic Fourier descriptors. The outline of this method used is shown in Fig. 1.

To describe the four varieties of Mokara based on the outlines of sepals and petals, the data were subjected to principal component analysis (PCA). Scatter plot and box plots showing the variations among varieties were also generated. One way analysis of variance (Kruskall Wallis) and Dunn's Multiple Range Test were performed using the scores of the components as characteristics of sepals and petals shape.

The lip or labellum of the flower was also used to describe variations among the different varieties of Mokara using the landmark-based method using 15 landmarks. The location of the landmarks used is shown in Fig. 2. The locations of these landmarks are homologous to all varieties of Mokara. These anatomic landmarks transformed to Cartesian coordinates were then analyzed by generalized orthogonal least-square procedure to separate the shape from the size component. Mean shape and relative warps were then calculated. Consensus configurations of the lip or labellum for all varieties were obtained using a scale a = -1. The = -1 were used to emphasized small scale variations (Hammer *et al.,* 2001) of the lip shape among the different orchid varieties. Deformation grids of the relative warps were taken using + and – amplitudes to graphically portray the patterns of shape variations among orchid lips. Scatter plot were then generated using the relative warp scores. The significant similarities and differences of lip shape among the different varieties were then showed by the convex hulls. The outline of this method of analysis is shown in Fig. 3.

RESULTS

Outline analysis: The use of elliptic Fourier descriptors captured the shapes of the three sepals (left, right & center) and two petals (left & right). Their standardized elliptic Fourier coefficients were calculated and these values were used to reconstruct the mean shapes of the 3 sepals and 2 petals (Fig. 4). Results showed that the shapes of sepals and petals between the four (4) varieties of Mokara varied widely. Within varieties however, both the left and right sepals and petals were symmetrical in shapes. The labellum was also observed to differ among the varieties examined.

To describe the nature of variations observed within and between the varieties of the orchids, PCA was done. The contributions of each component to sepal and petal shape variations are shown in (Tables I & II). The differences among left, right and center sepals were summarized in three effective principal components, which accounted for 94.49%, 95.13 and 96.73% variations respectively (Table I). The variations among left and right petals were summarized in three and two effective principal components, which accounted for 95.52% and 96.87%, respectively (Table II).

To illustrate the variations as contributed by each of the principal components, reconstruction of the sepals and petals was done. Results showed contour overlaps indicating variations among the different varieties of Mokara (Fig. 5). PC1 describes contour overlaps indicating that the sepals and petals vary in length-width ratio. The minor contour overlaps seen in PC 2 and PC3 detects the minor variations, which could not be detected by merely comparing the structures using human visual judgment. Further graphic descriptions of the variations in shapes are shown in Fig. 6 and 7. The box plots of the principal component scores in Fig. 6 further illustrate how the variations in the different varieties are described. Between Mokara Kultana Gold and Mokara Sayan Gold slight variations in shape was observed but these two varieties vary greatly from varieties Mokara Salaya Red and Mokara Kultana Red. This was further illustrated by the scatterplots in Fig. 7, where separations of the convex hulls were prominent between Mokara Salaya Red and Mokara Kultana Red but not in Mokara Kultana Gold and Mokara Sayan Gold. Kruskal Wallis test of all the effective principal component of both sepals and petals were all extremely significant indicating significant differences in the sepals, petals and labellum of the four varieties of Mokara orchids (Table III).

Landmark analysis: Fig. 8 shows the results of the landmark analysis of the labellum of the four varieties of Mokara. All the four varieties differed greatly in the shape of their labellum. Mokara Salaya Red and Mokara Sayan Gold had narrow base, while Mokara Red and Mokara Kultana Gold had wide base. The tip and the curvature at the sides of the labellum of varieties also differed in shape. Relative warp analysis further illustrates how the four varieties were differentiated by the shapes of the labellum. As illustrated by the relative warp grids, variations occur at the apex, the base and the sides of the labellum (Fig. 9).

The use of the two GM methods, the Fourier analysis of shape and the landmark analysis, were both effective in detecting variations among the four varieties of Mokara.

Fig. 1: Outline of elliptic fourier analysis of shape

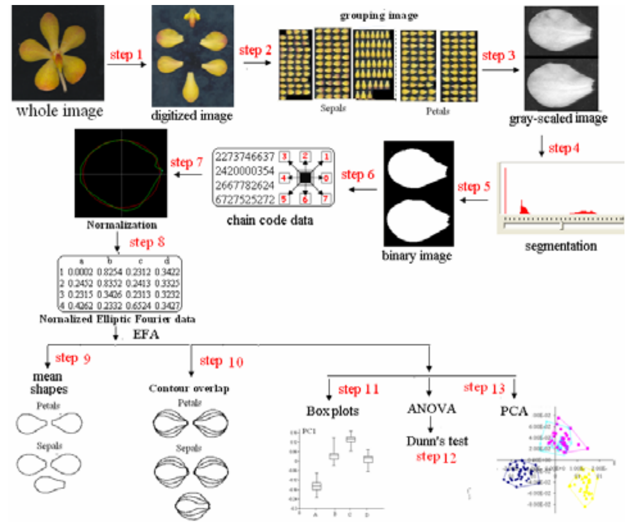


Fig. 2: Location of Landmarks at the labellum of the four (4) varieties of Mokara. (A). Mokara Salaya Red, (B). Mokara Kultana Gold, (C). Mokara Red and (D). Mokara Sayan Gold

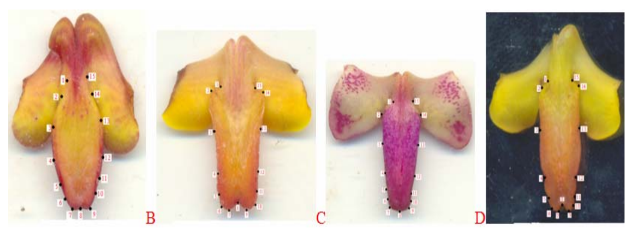
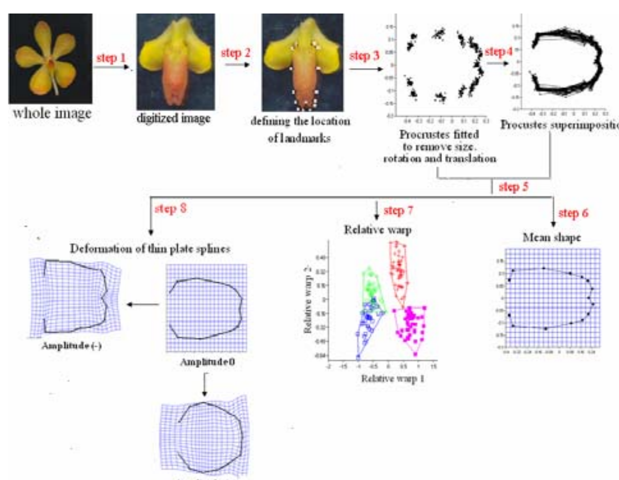


Fig. 3: Outline of landmark – based analysis



Variations among sepals and petals and the labellum revealed that these structures can also be used to discriminate the different varieties of Mokara. Discrimination among the varieties of Mokara could be achieved by using the sepals (left, right & center), petals (left & right) as well as the lip or labellum of the flower.

Fig. 4: Mean shape of sepals and petals of the four varieties of Mokara

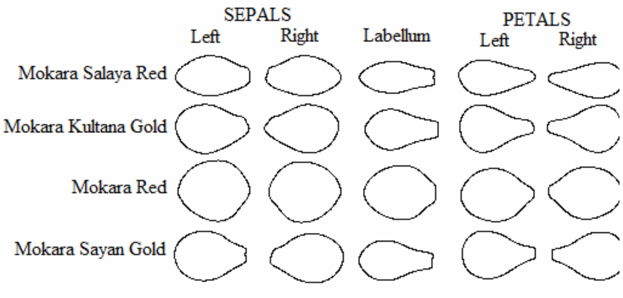


Fig. 5: Reconstructed image showing contour overlaps indicating the contributions of the effective principal components to the shape variations of petals and sepals of the four (4) Mokara varieties

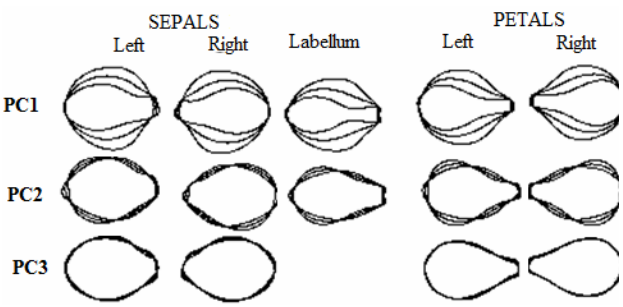
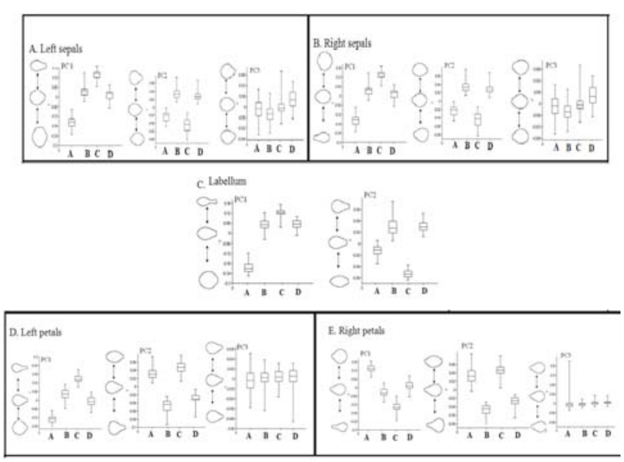


Fig. 6: Box plot graphs of the effective principal components of sepals and petals of the four (4) varieties of Mokara. (A). Mokara Salaya Red, (B). Mokara Kultana Gold, (C). Mokara Red and (D). Mokara Sayan Gold



However, each method has corresponding limitations as well as advantages as revealed by the study. In using the landmark-based method, the landmark must be manually defined using an image processing software which requires that time be devoted in the process.

In landmark based GM, all the data used in a single analysis must be based on the same set of landmarks, which implies that structures or specimens with missing landmarks, because they are broken or poorly preserved must be either eliminated from the analysis or the missing landmark be eliminated from the dataset so that all the specimens can be included in the analysis.

Fig. 7: Scatterplots of principal component scores delineating the four varieties of Mokara

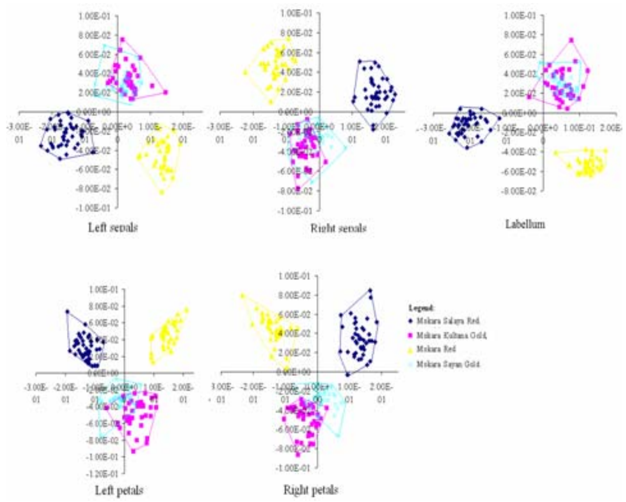


Fig. 8: Mean shapes of the labellum of the four (4) varieties of Mokara varieties

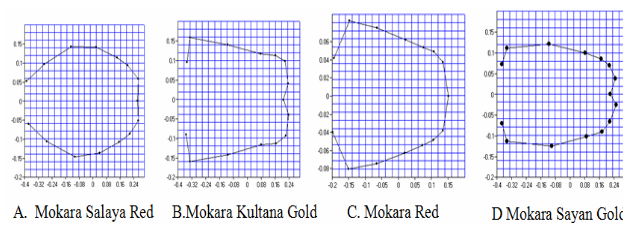


Fig. 9: Plots of relative warps (a) 1 and 2 (b) 1 and 3 and (c) 2 and 3 of the 4 Mokara varieties. The center landmark configuration illustrates the mean labellum shape; the other configurations illustrate the change in shape represented by movement along each axis in the direction indicated by the arrow

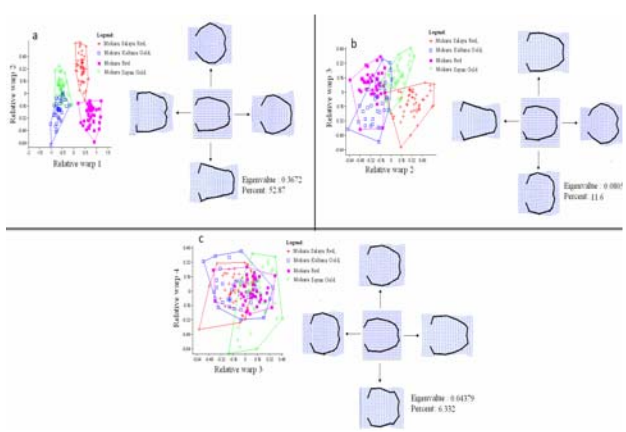


Table I: Eigen values and the contributions of the effective principal components to the shapes of sepals of the four (4) varieties of Mokara

Components	Left Sepals			Right Sepals			Center Sepals		
	Eigenvalue	Proportion	Cumulative	Eigenvalue	Proportion	Cumulative	Eigenvalue	Proportion	Cumulative
1	121.43	93.72	83.72	131.18	84.67	84.67	110.80	82.63	82.63
2	12.75	8.79	92.51	13.09	8.45	93.12	17.13	12.77	95.41
3	2.86	1.97	94.59	3.11	**2.01**	**95.13**	1.77	1.32	96.73
Total Variance	145.04			154.92			124.08		

Table II: Eigen values and the contributions of the effective principal components to the shapes of petals of the four (4) varieties of Mokara

Components	Left Petals			Right Petals		
	Eigenvalue	Proportion	Cumulative	Eigenvalue	Proportion	Cumulative
1	102.38	79.32	79.32	166.94	90.28	90.28
2	17.86	13.84	93.16	12.18	6.59	96.87
3	3.04	2.35	95.62			
Total Variance	129.06			184.89		

Table III: One way ANOVA (Kruskal-Wallis) of the effective components of sepals and petals of the four (4) Mokara varieties

Components	Left Sepals	Right Sepals	Center Sepals	Left Petals	Right Petals
1	129.35***	131.64***	122.36***	131.64***	138.43***
2	123.49***	127.52***	129.69***	127.52***	128.96***
3	39.07***	41.49***		41.49***	30.41***

***- P value <0.0001-extremely significant

The results of the current study however have minimized all these concerns as there were no missing data that were observed as the homologous landmarks were all identified in the samples examined. Likewise, the number of points used in the outline analysis is more than enough to capture the shapes of the structures. It can therefore be concluded from this study that both methods were able to describe shape variations in the sepals, petals and labellum among the four varieties and that the two methods of geometric morphometrics are very useful in understanding shape variations in the Mokara orchids.

DISCUSSION

According to Adams *et al.* (2004), sufficient number of landmarks may not be available to capture the shape of the structure, that is, there may be large region of a structure where no biologically meaningful points can be identified. Furthermore, important shape differences may also be located in the regions between landmarks thus variations in the said regions may not be detected. Results of the study have shown the importance of elliptic Fourier and landmark-based to evaluate quantitatively the petal, sepal and labellum shapes of four varieties of Mokara. Using principal component scores obtained from standardized elliptic Fourier descriptors, it can be observed visually how each principal component affects the shape by drawing the contours under particular score value conditions, thus demonstrating that the principal component scores can be used as new shape characteristics of Mokara petals and sepals. Elliptic Fourier descriptors and principal component analysis (EF–PCA) have accurately detected small shape variations and evaluate the shapes of the petal and sepal independently of size thus can be considered advantageous because human visual judgment of shape of the flower is often deceived and misled by size factors.

Results of landmark analysis have also shown that the labellum shape can also be differentiated between Mokara varieties. The procedure of general procrustes analysis eliminates non-shape variation in configurations of landmarks by superimposing landmark configurations using least squares estimates for translation and rotation parameters thus the only remaining difference between objects is their shape permitting for mulivariate statistical analysis and a straightforward way to visualize the corresponding shape changes in the labellum. The application of landmark-based analysis of GM therefore is a a good method of describing and analyzing shape changes in the labellum of Mokara orchids.

The differences observed in the four varieties of Mokara can be a reflection of their genetic differences. This is not surprising since Mokara is a product of trihybridization. In nature, the evolution of floral characters was hypothesized to be under pollinator-mediated selection (Darwin, 1862). Pollinators influence the evolution of a variety of floral traits, such as corolla size (Campbell, 1989; Galen, 1989; Campbell *et al.*, 1991, 1996, 1997; Herrera 1993a; Conner *et al.*, 1996a; Caruso, 2000), stigma exsertion (e.g., Conner *et al.*, 1996b), nectary–stigma distance (Johnston, 1991), flower stalk length (e.g., O'Connell & Johnston, 1998; Maad, 2000), spur length (e.g., Herrera, 1993a; Maad, 2000), flowering date (Campbell, 1989; Johnston, 1991; Wide´n, 1991; Kelly 1992; Go´mez, 1993), corolla color (Campbell *et al.*, 1997; Nagy, 1997; Go´mez, 2000) and corolla shape (Herrera, 1993a b; Nagy, 1997; Galen & Cuba, 2001). As to the functional significance of the variations observed, flower traits may have strong fitness consequences as reproductive success is being associated with specific flower shapes. Flower shapes are associated with reward (nectar & pollen) production (Ashman Stanton, 1991; Campbell *et al.* 1991; Cohen & Shmida, 1993; Møller, 1995; Smithson & Macnair, 1997; Neal *et al.,* 1998; Blarer *et al.,* 2002; Armbruster *et al.,* 2005; Fenster *et al.,* 2006; Boisvert *et al.,* 2007; Makino & Sakai, 2007) and determines the preference pattern of important pollinators (Lehrer *et al.,* 1995; Møller, 1995; Rodri´guez *et al.,* 2004; Go'mez *et al.,* 2008a & b; Gong & Huang, 2009). These natural selection events have resulted to the generation of new varieties and species in nature. What natural pollinators have influenced to the evolution of complex floral traits however is no different from the artificial breeding of several species of orchids to generate new varieties as exemplified by Mokara orchids. The mechanisms involved are not different except that in nature, the generation of new flower traits resulting from exchange of genes are facilitated by natural pollinators, while in artificial breeding, the traits of interest are selected to generate new floral traits.

There is however, a need for additional studies especially a detailed investigation of the complex flower traits on several varieties of the orchids using the tool of geometric morphometrics. Investigating the pattern of variation in flower shape, its heritability, the selection occurring on it and the local adaptation associated with it may be able to identify the factors driving flower shape evolution thus will be of big help to breeders in selecting for appropriate floral traits in producing new varieties.

In conclusion, this study shows the importance of GM in describing shapes of petal, sepal and labellum of Mokara orchid varieties. Both elliptic Fourier and landmark-based analyses require a dimension technique in the form of PCA to summarize independent shape characteristics. These results will help in the classification and discrimination of the varieties based on the shapes of petals, sepals and labellum.

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