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Relative warp analysis of head shape variations in *Nephotettix virescens* (Distant) (Homoptera: Cicadellidae) infesting rice types with different genes for resistance

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ABSTRACT

Head shape differences were examined in both sexes of the green leafhopper *Nephotettix virescens* (Distant) infesting a susceptible variety TN1, and rice varieties with specific genes for resistance TAPL (*Glh6*), Ptb8 (*Glh4*), MK (*Glh7*), and IR8 (*Glh3*). Differences in the shapes of the heads were qualified using advances in image analysis and geometric morphometric analysis. A total of 18 homologous landmarks and 2 pseudo-landmarks were digitized from images of dissected head parts of the samples using the *ScionImage* software. Then, the x and y coordinates of the landmarks were examined using relative warp analysis and principal component analysis. Results of the Kruskal-Wallis test (non-parametric ANOVA) of the head shape descriptors showed clear-cut divergence in the shapes of the heads of some green leafhoppers infesting resistant rice types (P<0.001). In the context of evolution, such observation shows that ecological divergence following host shifts may also be an important factor in the diversification of lineages of herbivorous insects. Such host races can thus serve as models to test hypotheses about the factors driving local specialization that can lead to reproductive isolation and speciation.

Keywords: Geometric morphometrics; Landmarks; Principal component analysis.

INTRODUCTION

Morphometrics is the study of shape variation and its covariation with other variables. This is the statistical study of biological shape and shape change. Its richest data are landmark points that have biological names as well as geometric locations (Bookstein, 1991; Strauss and Bookstein, 1982; Rohlf and Marcus, 1993). In the past decade, new methods for the analysis of biological shape have been elucidated and applied to evolutionary inquiry in a number of fields. Collectively, this new set of methods for analyzing landmark-based data is referred to as geometric morphometrics. Geometric morphometrics may be visually depicted in a Cartesian plane using 2-dimentional spaces or 3-dimentional spaces. This technique has been used to solve problems in ontogeny, phylogeny, and other problems related to ancestry including functional morphology. We used this technique to determine 2-dimentional form or shape difference within and between individuals of the green leafhopper *Nephotettix virescens* (Distant, 1908), an insect pest considered one of the most important *Nephotettix* species attacking rice in South and Southeast Asia (Claridge, 1979; Heinrichs and Rapusas, 1983; De Long, 1965; Karim, 1978; Ruangsook, 1986; Taulu et al., 1987; Hibino, 1987; Siwi et al., 1987). The evolution of populations of this pest that adapted to the resistant varieties without biological adverse effects to them has been the object of studies of many researchers. Thus this study was conducted to examine whether variability in fundamental features such as the shape of the head of this species of green leaf hopper could help us explore processes of selection and adaptation leading to selection of virulent of *N. virescens* threatening the value of resistance in cultivated rice.

MATERIALS AND METHODS

Head shape differences were examined in both sexes of the green leafhopper *Nephotettix virescens* (Distant, 1908) (Homoptera: Cicadellidae) infesting a susceptible variety TN1, and rice varieties with specific genes for resistance TAPL (*Glh6*), Ptb8 (*Glh4*), MK (*Glh7*), and IR8 (*Glh3*).

Geometric morphometrics analysis was used to capture the precise body form of the individual samples by land marking (Bookstein, 1991; Strauss and Bookstein, 1982; Rohlf and Marcus, 1993; Adams, 1999). A total of 18 homologous landmarks and 2 pseudo-landmark points were selected for the analysis of the head shape of sexes of *N. virescens* (Fig. 1). These landmarks were digitized from images of dissected head parts of the samples using the Scion Image software (www.scioncorp.com/pages/scion_image_windows.htm.). This software produces corresponding values to x and y coordinates in each designated landmark points. Shown in table 1 is a list of the anatomical description corresponding to the landmark.

The relative warps are the principal components of a set of thin-plate spline transformations. It provides a scatter plot that shows the similarities and differences between compared groups. The relative warps are themselves transformations, and can be visualized with grid deformations (Hammer et al., 2001). Principal components analysis (PCA) is a method that produces hypothetical variables or components, accounting are linear combinations of the original variables. This is a method of data reduction that in well-behaved cases makes it possible to present the most important aspects of a multivariate data set in two dimensions, in a coordinate system with axes that correspond to the two most important (principal) components. In other words, PCA represents a way of projecting points from the original, high-dimensional variable space onto a two-dimensional plane, with a minimal loss of information (Hammer et al., 2001). Kruskal-Wallis test was used to analyze whether or not the species differ significantly with regards to the head shape.

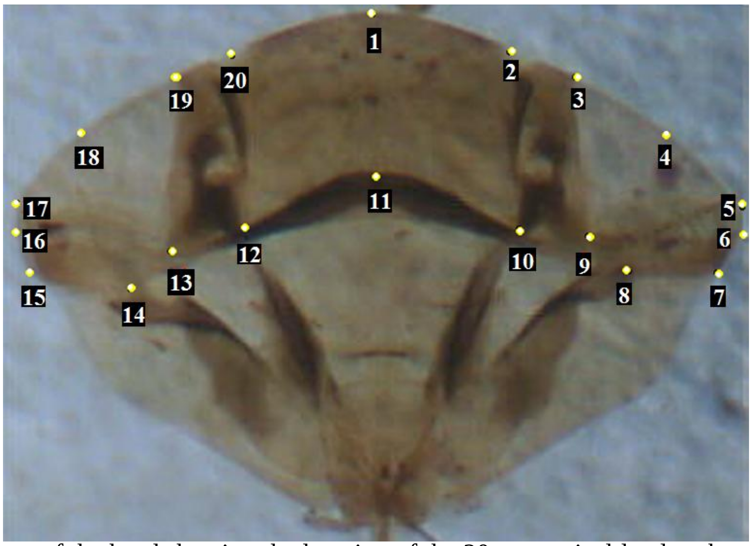

Fig. 1: Digitized image of the head showing the location of the 20 anatomical landmarks.

Table 1: Description of each landmark assigned to both left and right literalities of the head.

LANDMARK	ANATOMICAL DESCRIPTION	LANDMARK	ANATOMICAL DESCRIPTION
1	apex of vertex	11	base of vertex
2	right antero-lateral part of vertex	12	postero-lateral of left vertex
3	right antero-lateral part of the eye	13	posterior part of the left eye
4	right medial part of the eye	14	left postero-lateral part of gena
5	right lateral-most region of the eye	15	left postero-medial part of gena
6	right antero-lateral part of gena	16	left antero-lateral part of gena
7	right postero-medial part of gena	17	left lateral-most region of the eye
8	right postero-lateral part of gena	18	left medial part of the eye
9	porterior part of right eye	19	left antero-lateral part of the eye
10	postero-lateral part of the right vertex	20	left antero-lateral part of vertex

RESULTS AND DISCUSSION

Relative warps analysis was used to obtain a scatter plot that shows the similarities and differences between groups, as well as the components that give more contribution to variation. It was also utilized to obtain figures that allow visualization of the deformations that may occur.

Using Relative Warps Analysis (Figure 2), it was observed in the graph that two groups of resistant female *N. virescens* were formed along x axis of the Relative Warp 1 in the scatter plot, the first group are those species of *N. virescens* infesting TN1, TAPL, PTb8 and MK while the second group are species infesting *IR8*. This variation can be accounted for in the inward bending in the right postero-medial and postero-lateral parts of gena as well as in the left postero-medial and postero-lateral parts of gena.

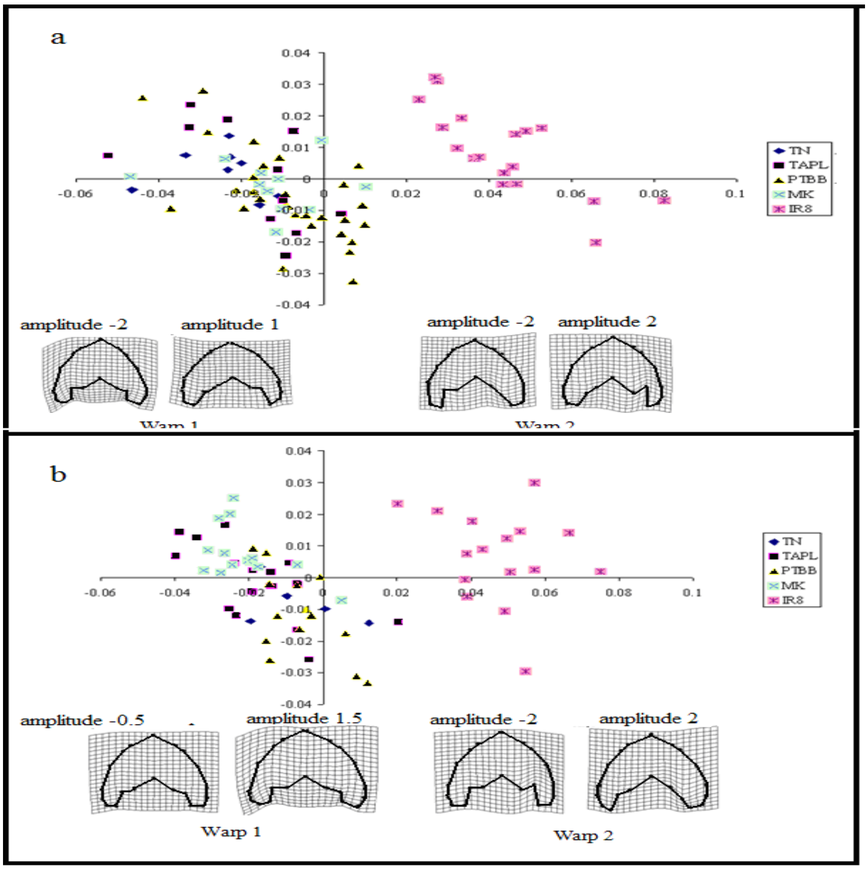

Fig. 2: Relative Warp Analysis ordination diagram and respective shape changes of the heads of resistant female (a) and (b) *N. virescens* associated with individual axes.

Figures 3 and 4 show the Relative Warp analysis ordination diagram and respective shape changes of the heads of resistant male *N. virescens* associated with individual axes. The first relative warp accounted for most of the morphological variation in the data set that result in the formation of two groups along the x axis. The first groups are those species of *N. virescens* that infest TN1, TAPL, PTb8 and MK while the second groups are the species that infest IR8. The grouping is due to the inward bending of the right postero-lateral part of gena and posterior part of the right eye as well as the outward bending of the right postero-lateral part of the vertex. Furthermore, it also described the outward bending of the left postero-lateral part of vertex and inward bending of the following landmarks; left posterior part of the eye, left postero-lateral part of gena and left postero-medial part of gena.

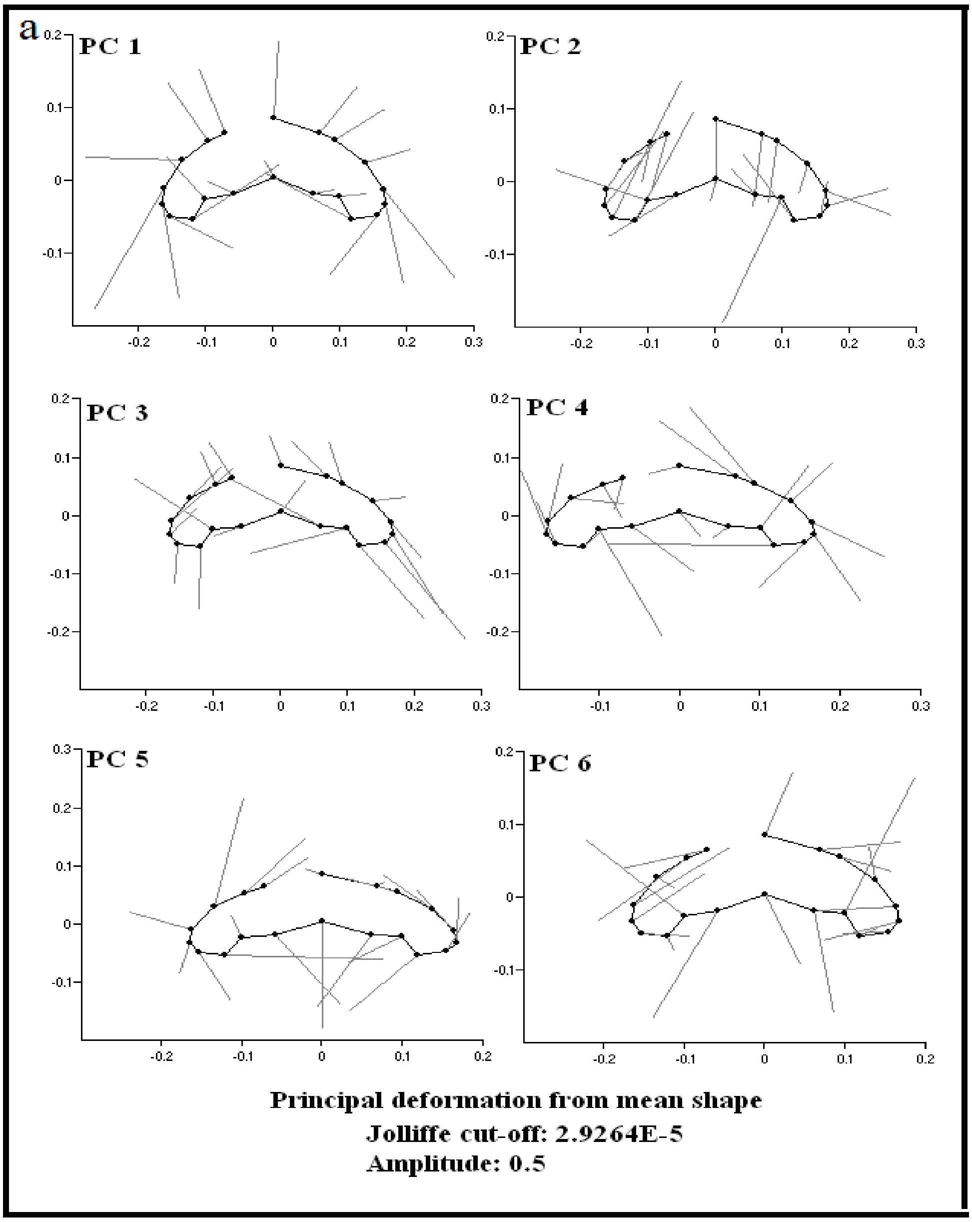

Fig. 3: Principal deformation from mean shape of the head of male *N. virescens.*

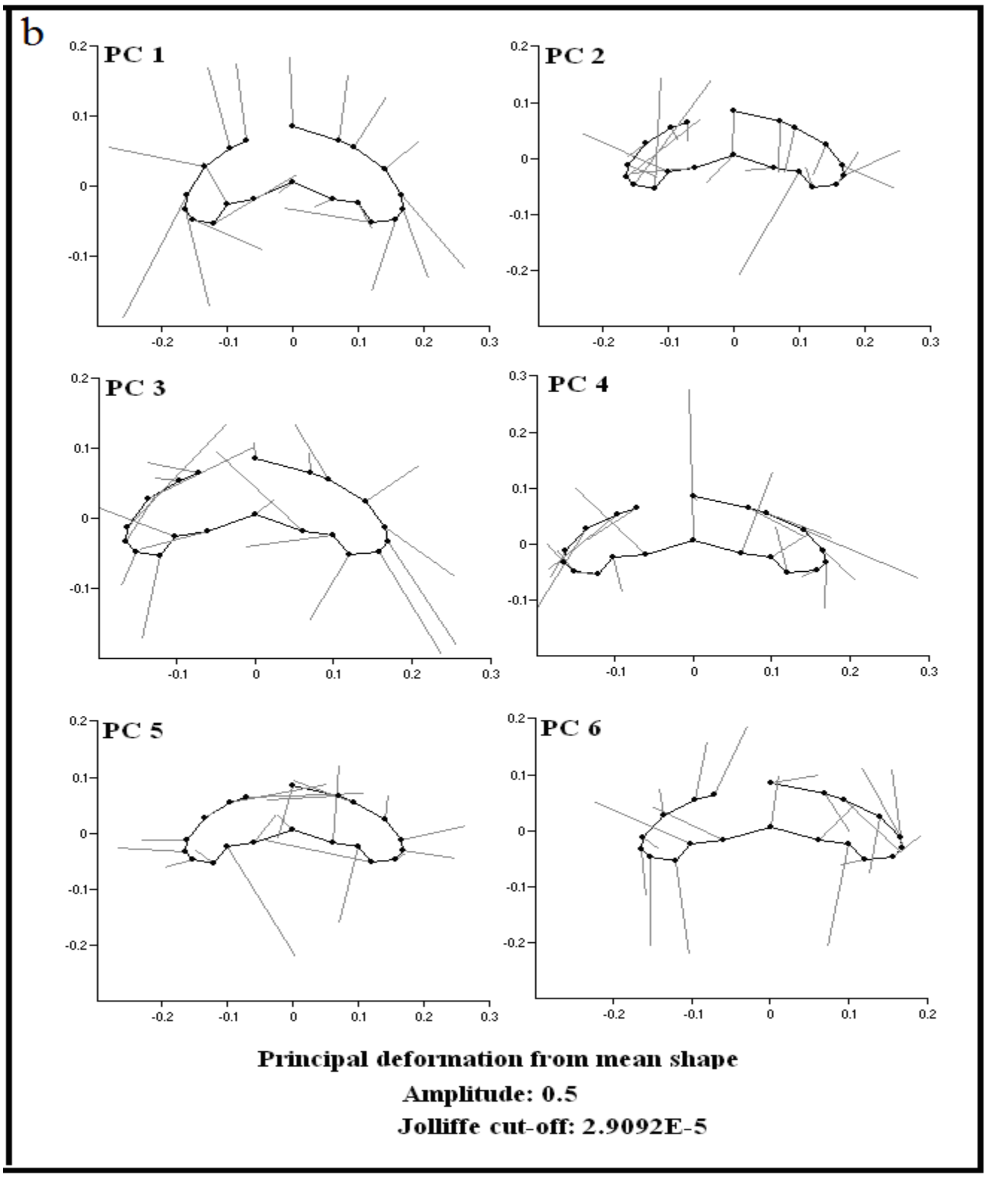

Fig. 4: Principal deformation from mean shape of the head of female *N. virescens.*

Principal Component Analysis (PCA) of Landmark Coordinates

Result of principal component analysis (PCA) shows the proportion of variation of the head shape of the resistant female and male *N. virescens* (Table 3). Each component reflects their corresponding Eigen values and % variance that provides the explanations between two axes (x and y coordinates). Decreasing value of percent variance is observed as the component goes to the next higher component.

Table 2. Proportion of variation of the head shape of male resistant *N. virescens*.

MALE			FEMALE		
PC	Eigen Values	% Variance	PC	Eigen Values	% Variance
1	0.000816336	48.818	1	0.000873501	52.544
2	0.00019801	11.841	2	0.000189205	11.381
3	0.000160169	9.5782	3	0.000130761	7.8657
4	8.7740E-05	5.2469	4	7.3303E-05	4.4094
5	6.2986E-05	3.7666	5	5.7578E-05	3.4635
6	4.8829E-05	2.92	6	5.6944E-05	3.4254
7	4.1186E-05	2.4629	7	3.7729E-05	2.2695
8	3.7230E-05	2.2264	8	3.4727E-05	2.0889
9	2.9496E-05	1.7639	9	2.9137E-05	1.7527

Table 3: Descriptions of the shapes of the head as revealed by PCA.

	MALE	FEMALE
PC1	Marked deformation in the following landmarks: upward elongation in the apex of vertex; elongation directed towards lower right in the right lateral most region of the eye, left postero-lateral part of gena and left antero-lateral part of gena; elongation towards lower left were observed in right postero-medial part of gena and left lateral most region of the eye as well as elongation towards upper left was observed in the right postero- lateral part of gena; downward elongation in right antero-lateral part of gena and left postero-medial part of gena were also noted,	The elongation in the apex of vertex is directed upward while there is marked elongation noted in the right lateral most region of the eye which is both directed towards lower right. Elongation in lower left was observed in the right postero-medial part of gena and left lateral most region of the eye while downward elongation was seen in right antero-lateral part of gena and in the left antero-lateral part of gena.
PC2	Elongations toward upper right were observed in left posterior part of the eye, left postero-lateral part of gena and left postero-medial part of gena. Elongation towards lower left in right posterior part of the eye and towards upper left in left posterior part of the eye was also observed	Elongation was observed in the left posterior part of the eye and left postero-medial part of gena being directed towards upper right and in right postero-lateral part of gena and right posterior part of the eye which are directed towards upper and lower left respectively.
PC3	The parts of the head that showed deformation were seen in the right antero-lateral part of gena, right postero-medial part of gena, right postero-lateral part of gena, their elongations were all directed towards upper right. Elongation towards upper left were noted in right postero-lateral part of vertex and left posterior part of the eye and towards lower left in the right posterior part of the eye.	Elongation towards lower right was observed in the postero-lateral part of vertex and in the left posterior part of the eye. The right antero-lateral part of gena and left lateral most region of the eye are directed towards upper and lower left respectively.
PC4	Landmark 13 is elongated towards lower left and elongations toward upper right were observed in landmarks 2 and 3.	An elongation was observed at the base of the vertex (upward elongation), right postero-lateral part of vertex (upper right elongation) and left postero-lateral part of vertex (upper left elongation).
PC5	Showed evidence of elongation directed towards upper right in the left postero-lateral part of gena and left medial part of the eye and towards lower right in left postero-lateral part of vertex. It was also observed that elongations in the right postero-lateral part of gena and right posterior part of the eye were directed towards lower left. The base of vertex also revealed downward elongation.	Elongation was observed in the apex of vertex (upward elongation). The right postero-lateral part of vertex and the base of vertex are directed towards lower left.
PC6	The right postero-lateral part of vertex and the base of the vertex had elongations directed towards lower right and upper right in the right posterior part of the eye. Elongations in lower left and upper left in the left postero-lateral part of vertex and left posterior part of the eye respectively.	An upper right elongation was observed in right posterior part of the eye, also elongation towards upper left at the base of vertex and lower left in the left postero-lateral part of vertex and left postero-lateral part of the eye.

Result of the Kruskal-Wallis test of the head shape descriptors of the populations (Table 4) showed clear-cut divergence in the shapes of the heads of green leaf hoppers (female and male) infesting IR8 rice (P<0.001).

Table 4: Results of the Kruskal-Wallis Test (Nonparametric ANOVA) for the significant differences of the means between two populations of Resistant strain *N. virescens.*

SEX	KW	P-VALUE	REMARKS
Male	24.512	<0.001	extremely significant
Female	44.374	<0.001	extremely significant

Table 5: Results of the Dunn's multiple comparison tests for the male and female heads of *N. virescens*. The KW statistic is found below the empty diagonal spaces. The P-values are above the diagonal spaces.

	MALE					FEMALE				
	TN1	**TAPL**	**PTB8**	**MK**	**IR8**	**TN1**	**TAPL**	**PTB8**	**MK**	**IR8**
TN1		<0.001	ns	<0.05	ns		<0.05	ns	<0.001	ns
TAPL	45.614		ns	ns	<0.01	-28.979		ns	ns	<0.001
PTB8	23.905	-21.708		ns	ns	-22.4	6.579		ns	<0.05
MK	31.705	-13.909	7.799		ns	-42	-13.021	-19.6		<0.001
IR8	13.694	-31.919	-10.211	-18.01		1.1	30.079	23.5	43.1	

It can be seen from the results that geometric morphometrics is effective in examining variability in fundamental features such as the shape of the head in populations of green leaf hopper *N. virescens*. The relative warps analysis and cluster analysis of shapes data revealed the single most important trend dividing the species into two groups along the x axis in figures 2 and 3 (group 1- species infesting TN1, TAPL, PTb8, MK; group 2- species infesting IR8), the inward bending in the right postero-medial and postero-lateral parts of gena as well as in the left postero-medial and postero-lateral parts of gena (female resistant strain). In the male resistant strain, the variation is attributed to the inward bending of the right postero-lateral part of gena and posterior part of the right eye as well as the outward bending of the right postero-lateral part of the vertex. Furthermore, it also described the outward bending of the left postero-lateral part of vertex and inward bending of the following landmarks; left posterior part of the eye, left postero-lateral part of gena and left postero-medial part of gena. Results of the Kruskal-Wallis test (non-parametric ANOVA) of the head shape descriptors of the populations showed clear-cut divergence in the shapes of the heads of green leaf hoppers infesting IR8 rice ($P<0.001$). Many changes in the ecological and physiological traits of the species are frequently followed by subtle changes in its morphological characteristics (Bey-Bienko, 1958). Morphology is an end-product of physiological activities initiated by the genome and modified by the environment. A change in physiology at the immature stage would result in a change in morphology at the adult stage. A closer look at the present study shows that the host plant is one of the important factors that influence the morphological differences in infesting populations.

In the context of evolution, such observation shows that ecological divergence following host shifts may also be an important factor in the diversification of lineages of herbivorous insects. Such host races can thus serve as models to test hypotheses about the factors driving local specialization that can lead to reproductive isolation and speciation.

CONCLUSION

It can be concluded from the results of the image analysis of the heads of the green leafhopper *N. virescens* that variations within and between populations can be described. Geometric morphometrics is an effective tool in describing shape variations in the green leafhopper. Likewise, a closer look at the present study shows that the host plant is one of the important factors that influence the morphological differences in populations of this insect pest.

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