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Describing compartmentalization in the hind wing of the hispid beetle *Brontispa longissima*

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ABSTRACT

This study was conducted to determine the possible number of developmental modules defining the shape of the hind wing of coconut hispid beetle B. longissima. Geometric Morphometric analysis (GM) was used to describe the shape of the wings summarized via Procrustes analysis. A total of 180 landmark points trace and outline the margins of the wings as well as the major veins. Modularity and Integration Analysis (MINT) *software was used as a tool to test a priori models of variational modularity in multidimensional data. Thirteen a priori models of variational modularity in the GM hind wing data were tested using the γ* (Gamma*) test for goodness of fit (GoF) statistics by comparing the observed and expected covariance matrices. Results showed that both male and female coconut hispid beetle hind wing is organized into four modules, the partition of cubitus and media, radius and media, costa+subcosta and radius and cubitus and anal vein. Differences in ranks of the top 3 models were observed in male and female left wing and right wing. It was hypothesized to be caused by effects associated with the signalling sources for position and morphology of pattern elements which only covers short distances.*

Keywords: *Brontispa lonngissima*, hindwing, Integration, Modularity

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INTRODUCTION

Insect wings, one of the most important organs of insects, have large contribution for them to become the most prosperous biological community. Wing traits evolve rapidly to respond to various environmental conditions [1]. These wings expand insects' activity, distribution scope and at the same time increase their speed. And therefore, have large contribution to the unparallel success and wide diversity of insects [2]. Wing venation is species specific and is used taxonomically [3]. A number of studies published on compartmentalization in insect wings suggest that one or set of genes control wing development [4-6]. It has been hypothesized that subdivisions or compartments of insect wings correspond to a distinct cell lineages and domains of gene expression [7-9]. Each wing compartment is a potential candidate of being separate and distinct developmental module that is reflected in phenotype and genetic variation [9].

Modularity is related to the concept of "morphogenetic fields" (Gilbert *et al.*, 1996) for they are constituted by the localized developmental processes that take place within them, and to the concept of "morphological integration" wherein such modules are considered to be structural units that are internally integrated by developmental interactions [10]. Thus, modularity discusses a degree of evolutionary autonomy to the sets of traits integrating a module by allowing selection to optimize individual parts without interfering with others [11].

In this study, Modularity and Integration (MINT) analysis [11] was used to determine the autonomous unit of morphological variation that could be considered as developmental modules. It is to show that modules are considered as subsets of dimensions embedded in phenotypic space. This allows traits to be integrated into more than one module and suggest a natural approach for testing a priori hypothesis of modularity by fitting competing hypotheses to observed covariance matrices, searching for the best-supported causal explanation [11]. Hence, the objective of the study is to determine the possible number and pattern of developmental modules defining the hind wing of the hispid beetle *Brontispa longissima*. This study aims to delimit the spatial domain of developmental modules in the hind wing and to determine whether the compartments, even smaller parts of the wings could be considered as autonomous unit of morphological variation.

Understanding relationship between modules in the wings of the coconut hispid beetles can elucidate the underlying biological process of compartmentalization in the wings. Likewise, recognizing how covariation between modules can have substantial implications for understanding genetic variation and the potential of species for evolutionary change.

MATERIALS AND METHODS

Collecting and preparation of samples

Samples were collected and placed in a properly labeled container filled with 70% ethanol. Sex of samples was identified through visual inspection of genitalia under a stereo microscope.

The hind wings were detached and were mounted neatly in clean and clear glass slides and were properly labeled. Digital images were captured using Olympus E-410 DSLR Camera attached on a Leica Stereomicroscope.

Model Construction and Model Testing

A total of 180 points were used for outlining the shape, as well as the wing venation pattern of *B. longissima* using TPSdig2 version 2.17 [12]. After outlining, the TPS curve was then converted into landmark points (XY) using TPSutil (Rohlf, 2009) which served as the raw data for the analysis.

Wings of *B. longissima* possess five main vein stems: Costa (C), Subcosta (Sc), Media (M), Radius (R), Cubitus (Cu) and Anal Vein (A) (Figure 1). To investigate this, different hypothesis (Table 1) were formulated to test and determine whether the entire wing is a single module or whether the compartments are independent units.

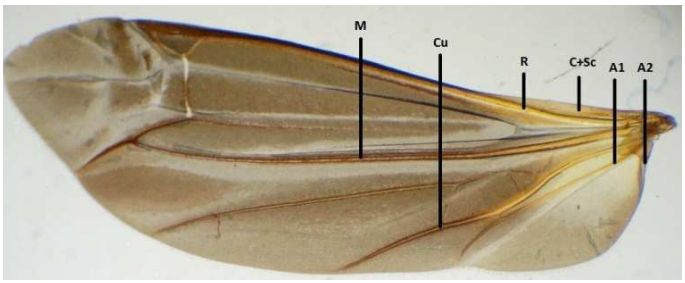

Figure 1. *B. longissima* hind wing showing veins that may serve as boundaries of the hypothesized developmental modules
Legend: C = Costa, Sc = Subcosta, R = Radius, M = Media, Cu = Cubitus and A = Anal Vein.

Modularity and Integration Tool (MINT) for Morphometric Data version 1.6 [11] was used to study modularity and integration in the hind wings of the selected population of *B. longissima*. MINT software calculates the matrix correlations between expected and observed covariance matrices. A total of 13 a priori models for the hind wings (Figure 2) were constructed with the help of model building tool option of the software. MINT assumes that the data have modular structure and that by positioning the entire data space into orthogonal subspaces, covariance matrices were then computed based on the modified data structures [13].

Table 1. A priori developmental modules of modularity tested in this study. Modules correspond to regions of the hind wing of coconut hispid beetle, *B. longissima* as hypothesized

MODEL	MODULES	DESCRIPTION	MODEL	MODULES	DESCRIPTION
H_0	0	Null model, Wings don't have modular structure	H_7	3	First module is bounded by Cu and M
H_1	5	First module is bounded by Cu and M			Second module is bounded by C+Sc, M and R
		Second module is bounded by M and R			Third module is bounded by Cu, A and wing posterior margin
		Third module is bounded by Cu and A	H_8	4	First module is bounded by Cu and M
		Fourth module is bounded by A and wing posterior margin			Second module is bounded by R and M
		Fifth module is bounded by C+Sc and R			Third module is bounded by C+Sc and R
H_2	4	First module is bounded by Cu and M			Fourth module is bounded by Cu, A and wing posterior margin
		Second module is bounded by C+Sc, R and M	H_9	3	First module is bounded by C+Sc, Cu, R and M
		Third module is bounded by Cu and A			Second module is bounded by Cu and A
		Fourth module is bounded by A and wing posterior margin			Third module is bounded by A and wing posterior margin
H_3	2	First module is bounded by M, Cu and A	H_{10}	4	First module is bounded by Cu, M and A
		Second module is bounded by C+Sc, R and M			Second module is bounded by R and M
H_4	3	First module is bounded by Cu, M, R and A			Third module is bounded by C+Sc and R
		Second module is bounded by C+Sc and R			Fourth module is bounded by A and wing posterior margin
		Third module is bounded by A and wing posterior margin	H_{11}	3	First module is bounded by Cu, R and M
H_5	4	First module is bounded by Cu, R and M			Second module is bounded by C+Sc and R
		Second module is bounded by C+Sc and R			Third module is bounded by Cu, A and wing posterior margin
		Third module is bounded by Cu and A	H_{12}	3	First module is bounded by Cu, M, A and wing posterior margin
		Fourth module is bounded by A and wing posterior margin			Second module is bounded by R and M
H_6	3	First module is bounded by Cu, M and A			Third module is bounded by C+Sc and R
		Second module is bounded by C+Sc, M and R	H_{13}	1	First module is bounded by C+Sc, R, M, Cu and A
		Third module is bounded by A and wing posterior margin			

Legend: C = Costa, Sc = Subcosta, R = Radius, M = Media, Cu = Cubitus and A = Anal Vein

The patterns of variational modularity were tested using γ* (Gamma) test for the Goodness of Fit (GoF) on the alternative a priori models to evaluate whether a proposed model or hypothesis is good enough to explain variation in the data set. Resulting P-values and γ*values depict associations within integrated sets of traits or variational module. A low (<0.05) P value, closer to zero, indicates that the models generated are significantly different from the observed data. The model is thus a poor fit and must be rejected. However, P-values greater than 0.05, (P>0.05) and approaching 1, correspond to low γ* values. This indicates a high degree of similarity between the proposed model and the observed data and thus the proposed model is accepted [11].

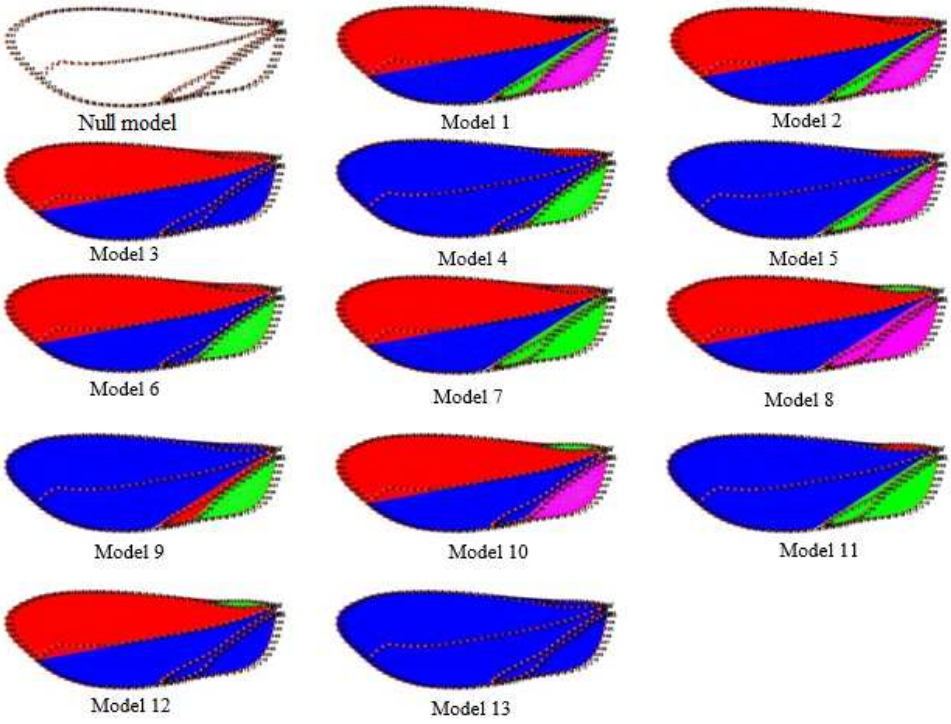

Figure 2. Models used in this study for the hind wing of coconut hispid beetle, *B. longissima*

RESULTS AND DISCUSSION

Based from the Monte Carlo test, the resulting P-values and gamma (γ*) values (Table 2) which depict associations within integrated sets of traits or variation modules show the top three models in the compartmentalization of the hindwing of *B. longissima* (Table 2). While variations were observed in the ranking of the bet-fit model based on the the low gamma value model 9 was the common model for both sexes (Figure 3). Based from this model, the wing is partitioned into 4 modules bounded by major veins in the wings: (1) bounded by cubitus and media, (2) bounded by radius and media, (3) bounded by costa + subcosta and radius, (4) bounded by cubitus, anal vein and wing posterior margin. Each module consists of a unit that is tightly integrated internally but relatively independent from other modules. Integration within each module is defined as the cohesion among traits that results from interactions of biological process producing the phenotypic structures [14].

Results also show that the main veins serve as boundaries for each developmental module. The compartment boundary (veins) apart from being a delimiter between autonomous unit of developmental domains or modules also serves as an active center of integration, from which crucial patterning signal originate [15]. Signals that are said to be originating from compartments boundary constitute a direct connection between the developmental processes which is responsible for positioning the various veins [16].

Results in this study show a fair consistency in the best fit model and in the pattern of developmental modules which may imply that the wings of *B. longissima* are highly conserved and that both male and female follow the same patterns of development. Consistency also suggests that there is not much variation in the arrangement of developmental modules between male and female in the wings of *B. longissima*.

Table 2. Computed γ*- and P- Values for the left and right hind wings of male and *female B. longissima* (Only the top three (3) best fit models were tabulated)

SEX	WING	MODEL	RANK	γ*-VALUE	P-VALUE
MALE	LEFT	9	1	**0.409**	0.004
		8	2	0.428	0.905
		13	3	0.470	0.99
	RIGHT	13	1	**0.407**	0.988
		9	2	0.409	0
		4	3	0.419	1
FEMALE	LEFT	9	1	**0.420**	0.006
		13	2	0.428	0.944
		8	3	0.430	0.002
	RIGHT	9	1	**0.393**	0.142
		4	2	0.401	0.006
		13	3	0.409	1

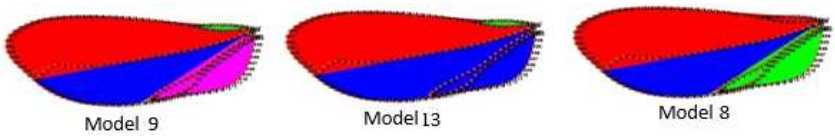

Figure 3. Top 3 best fit models for the hind wing of coconut hispid beetle, *B. longissima*. Model 9 showed consistency for male and female hind wings

However, the existence of other top alternative modules, as shown with the difference in ranking, may indicate evidence developmental interactions [17], phenotypic plasticity or factors that act during ontogenetic development [18-19]. The differences in the number of modules shown by the top 3 models could have a developmental basis. Lack of internal constraint is plausible where the position and morphology of each pattern element determined by signaling sources have effects extending only over short distances [20] since the signal does not appear to pass across the wing veins, or, there were lack of physical communication between them and/or from the wing-cell-specific genetic composition [21]. Nevertheless, this study is in conformity to a number of studies suggesting that insect wings are divided into compartments each of which is a separate developmental module. Compartments represent individual units of selection and that they are distinct units of selection subjected to different genetic control [22-25, 6, 15].

CONCLUSION

Results showed that the hind wings of the coconut hispid beetle, (*B. longissima*) is composed of 4 mutually exclusive and integrated developmental modules. These modules are also morphological units with clear spatial boundaries. These modules are suggested to be tightly integrated resulting from interactions of different biological processes.

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