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Describing compartmentalization in the Inner hind wings of three local populations of the coconut leaf beetle, *Plesispa reichei*

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Abstract

This study was conducted to describe compartmentalization in the inner wings of three local populations of two-colored coconut leaf beetle, *Plesispa reichei*. Modularity and Integration Analysis (MINT) tested 5 *a priori* models of variational modularity in multidimensional data. Using γ^* (Gamma*) test for goodness of fit (GoF) statistics comparing the observed and expected covariance matrices show that the inner wing is composed of 3 mutually exclusive and integrated developmental modules. One of the three populations however differ not only between the left and right wing but also based on the priority models of compartmentalization. While variations between populations were observed, results still confirm the idea that the modules observed in the wings are morphological units with clear spatial boundaries and are tightly integrated.

Keywords: Compartmentalization, developmental modules, integration, modularity, boundaries

1. Introduction

The insect wing is considered an important organ of insects contributing to their being most prosperous biological community [1]. It is designed for flight, but the shapes in this group of organisms have not converged into one optimal shape despite millions of years of evolution. It is also considered a subject of extensive analysis in a number of species, although knowledge of the genetic basis of its development is mainly restricted to the fruit fly *Drosophila melanogaster* [2]. Tremendous amount of variability in venation patterns such as folding and other characters such as shape, size and patterns of cell differentiation [3] underlie their adaptation to a variety of functions such as locomotion, defense, melanism and regulation of body temperature. These few characters are also considered basis for their classification in many species [4, 5] based on comparative qualitative description as analysis. Since wing traits evolve rapidly to respond to various environmental conditions and wing venation is species-specific, variations within the species can be explored by determining compartmentalization or the number of modules defining the morphological shape of the wing. Insect wings suggest that one or set of genes controls its development [6] thus subdivisions or compartments of insect wings differentiated by major veins may correspond to distinct cell lineages and domains of gene expression [7, 8, 9]. We therefore examined this in one of the most damaging invasive insect pest of coconuts, the two-colored coconut leaf beetle, *Plesispa reichei* (Chapui) first described by Corbett [10] and is now considered a major pest of coconut palm nurseries in the Philippines, Indonesia and Thailand [11], Miri Sarawak [12]. Continuous hot weather and the consequential low natural enemy populations are considered major factors that lead to outbreaks [13].

In recent years, advances in biology, geometry, computer imaging, and statistics have contributed to a better description of variations advancing our knowledge of the quantitative nature of variations in living organisms such as the wing. For example, quantitative morphological data have been used to characterize developmental, genetic, functional and evolutionary modules in the wing of Odonata [14]. Modularity is related to the concept of "morphogenetic fields" 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 among traits but are relatively independent from other modules [15]. In this study, Modularity and Integration (MINT) analysis [16] was used to determine the autonomous unit of

morphological variation that could be considered as developmental modules in *P. reichei*. This method of analysis 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 observe covariance matrices, searching for the best-supported causal explanation [16]. This study was conducted to determine variability between populations of the past by looking at the possible number and pattern of developmental modules defining the inner wings of *P. reichei* and whether the compartments, even smaller parts of the wings could be considered as autonomous unit of morphological variation in three local populations of the insect.

2. Material and Methods

Samples of coconut leaf beetles (*P. reichei*) were randomly collected from different areas in Mindanao: Balo-i, Lanao del Norte, Pantao Ragat, Lanao del Norte and Wao, Lanao del Sur (Fig. 1) from March – April 2014. Samples were collected and

placed in a properly labeled container filled with 70% ethanol. The inner 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.

A total of 190 points were used for outlining the shape, as well as the wing venation pattern of *P. reichei* (Fig. 2) using TPSdig2 version 2.17 [17]. After outlining, the TPS curve was converted into landmark points (XY) using TPSutil [18] which will serve as the raw data for the analysis before loading to the MINT (Modularity and Integration Analysis Tool) software [16].

Wings of *P. reichei* possess five main vein stems: Costa (C), Subcosta (Sc), Media (M), Radius (R), Cubitus (Cu) and Anal Vein (A) (Fig. 2). 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.



Fig 1: Map of the Philippines showing the locations of sampling areas in Mindanao: Balo-i, Lanao del Norte, Pantao Ragat, Lanao del Norte and Wao, Lanao del Sur.

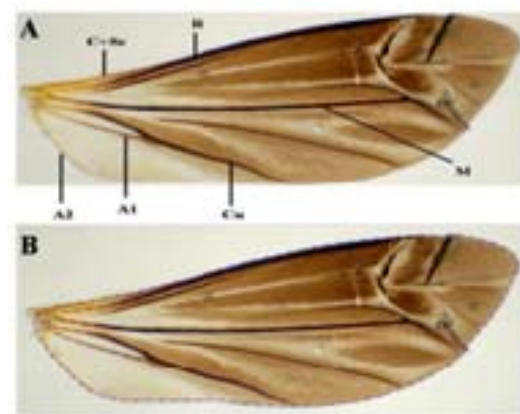


Fig 2: (A) Anatomical parts and (B) diagrammatic representation of coconut leaf beetle (*P. reichei*) inner wing indicating the landmarks outlined in this study. Legend: C=Costa, Sc=Subcosta, R=Radius, M=Media, Cu=Cubitus and A=Anal vein.

A total of 5 *a priori* models were constructed which generated 5 alternative models for the inner wings. This is already inclusive of the null model, which assumes the absence of modularity and integration. Each model hypothesizes a distinct modular structure caused by a specific functional or developmental mechanism. As shown in (Fig. 3), model 1 represented the null model; the second, third, fourth, fifth and sixth a priori models were set based on wing compartments and vein positions (Table 1). Defined models were then combined by the software to produce variational or alternative models for analysis.

Modularity and Integration Tool (MINT) for Morphometric Data version 1.6 [16] was used to test the acceptability of hypothesized modules. MINT software calculates the matrix correlations between expected and observed covariance matrices. MINT assumes that the data have a modular structure and that by positioning the entire data space into orthogonal subspaces, covariance matrices were then computed based on the modified data structures [19].

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Models with orthogonal covariance matrix were then combined and tested using the γ^* (Gamma*) test for goodness of fit (GoF) statistic. GoF computed matrix correlations between expected and observed covariance matrices (rM), and significance of rM values was tested following Monte Carlo randomization test with 1000 replications. Lower γ^* value simply indicates a high degree of similarity between the observed data and the proposed model. Meanwhile, a low (<0.05) P-value corresponds to large values of γ^* , indicating a large difference between data and model and thus a poorly fitting model [16]. The confidence interval for γ^* were obtained using jackknife resampling method [36] in which a randomly chosen subset of 10% of the specimens were dropped from each sample to produce 1000 subsamples, from which 95% confidence intervals were computed. Finally, a measure of model support called “jackknife support” was computed by counting the proportion of jackknife samples in which a model ranks first (i.e., has the lowest value of γ^*) [32].

Table 1: A priori developmental modules of modularity tested in the study. Modules correspond to regions of the inner wing of two-colored coconut leaf beetle, *P. reichei* as hypothesized.

Model	Modules	Description
H ₀	None	“Null” model, predicting absence of modular structure; all covariances are hypothesized to be zero.
H ₁	Single	The wing is considered as a single homogenous developmental module.
H ₂	Four	The first module is bounded by C+Sc and R. The second module is bounded by R and M. The third module is bounded by Cu and M. The fourth module is bounded by Cu, A, and wing posterior margin.
H ₃	Four	The first module is bounded by C+Sc, R and M. The second module is bounded by Cu and M. The third module is bounded by Cu and A. The fourth module is bounded by A and wing posterior margin.
H ₄	Five	The first module is bounded by C+Sc and R. The second module is bounded by R and M. The third module is bounded by Cu and M. The fourth module is bounded by Cu and A. The fifth module is bounded by A and wing posterior margin.
H ₅	Three	The first module is bounded by C+Sc, R and M. The second module is bounded by Cu and M. The third module is bounded by Cu, A, and wing posterior margin.

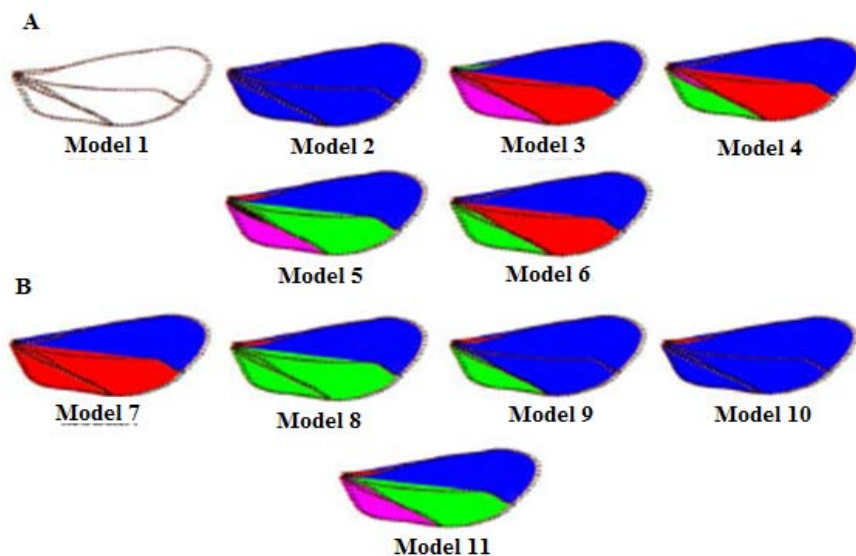


Fig 3: Models used in this study- (A) *A priori* / proposed models and (B) Nonhierarchical/ alternative models generated by MINT software.

3. Results and Discussion

Results showed that the best-fit model for the inner wings of *P. reichei* which was fairly consistent to all sampled populations was model 6 (Table 2). The model yielded a P-value greater than $\alpha=0.05$; this accepts the hypothesis that the

proposed model and the observed data are not significantly different. Model 6 also acquired the lowest (γ^* value), which qualifies it as a best-supported model among the five *a priori* models hypothesized for all samples tested.

Table 2: Computed γ^* - and P- Values for the left and right inner wings of *P. reichei* (Only the top three (3) best fit models were tabulated).

Population	Wing	N	Model	Rank	γ^* -value	p-value
Balo-i, Lanao del Norte	Left	30	6	1	0.13025	1
			4	2	0.13527	1
			3	3	0.13938	1
	Right	30	7	1	0.20421	1
			8	2	0.20564	1
			6	3	0.22996	1
Pantao Ragat, Lanao del Norte	Left	30	6	1	0.20466	1
			4	2	0.21309	1
			7	3	0.21495	1
	Right	30	7	1	0.26361	1
			6	2	0.27407	1
			4	3	0.28741	1
Wao, Lanao del Sur	Left	30	6	1	0.16815	1
			4	2	0.17549	1
			7	3	0.1955	1
	Right	30	6	1	0.16761	1
			4	2	0.17504	1
			7	3	0.19491	1

The best-fit model (Model 6) hypothesizes that each compartment represents distinct modules such that the genes controlling modules affect the developmental and genetic modularity of the wings. The model divides the wing into three distinct modules. The first module is bounded by Costa and Subcosta (C+Sc) vein, and encompasses the Radius and Media. The second module is bounded by Cubitus and Media

veins. The third module is bounded by Cubitus, Anal veins and the posterior margin of the wing (Fig. 4). Each of these modules 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 [20].

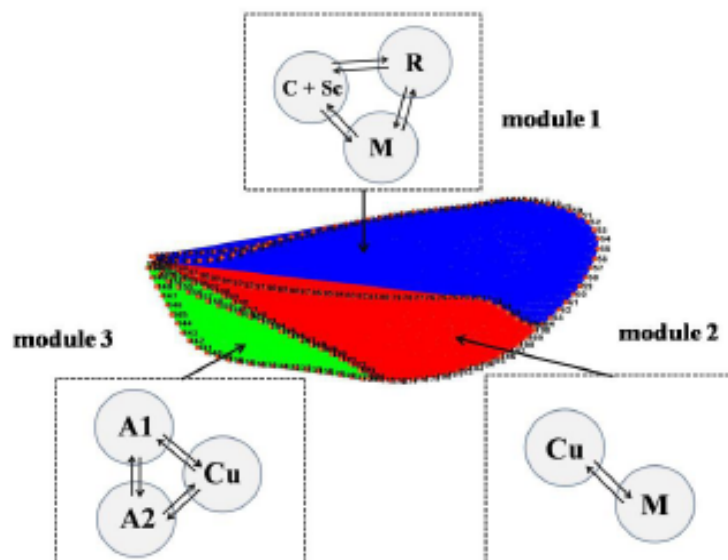


Fig 4: Hypothesized possible developmental modules of best fit Model 6 for the inner wing of two-colored coconut leaf beetle, *P. reichei*. Component parts within modules are interconnected by many interactions whereas there are fewer interactions between modules.

The existence of other top alternative modules, as shown with the difference in ranking in the three local populations may indicate evidence developmental interactions [21], phenotypic plasticity or factors that act during ontogenetic development [22]. 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 [23] 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^[24]. The current results also confirm a number of studies suggesting that the compartments, or even smaller parts of the wing, are autonomous units of morphological variation, and consequently, each of them is a separate developmental module^[1, 7, 14, 25, 26, 27]. Furthermore, the results are in conformity with a number of studies that each wing vein has its own identity yet interconnected to the others and that signals originating from compartment boundaries initiate regulatory interactions that subdivide the wing into series of sectors with discrete boundaries such that different sectors are distinguished by the expression of a different combination of genes and specific genes are activated at their boundaries to initiate vein formation^[28].

4. Conclusion

The results of the study show that the inner wings of *P. reichei* are composed of three mutually exclusive and integrated developmental modules. These modules are also morphological units with clear spatial boundaries. The observations of the existence of other alternative models indicate that the modules are tightly integrated resulting from the interactions of different biological processes. The variation in rank and type in the models between populations indicates population diversity in the species.

5. Acknowledgement

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