

Describing Developmental Modules in the Hind Wing of Rice Grasshopper, *Oxya sp* Using MINT Software

Kimverly Hazel I. Coronel, Mark Anthony J. Torres and Cesar G. Demayo

Dept. of Biological Sci., College of Sci. and Mathematics, Mindanao State Uni.-Iligan Institute of Tech., Tibanga, Iligan City, PHILIPPINES

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Abstract

Subdivisions of insect wings have attracted special attention due to its possible correspondence to distinct cell lineages and domains of gene expression. Hence, concept of modules comes into mind, which can also be viewed as morphogenetic field and units of gene regulation. This study was conducted to delimit the spatial domain of developmental modules in the fore wings of the selected population of Rice grasshopper, *Oxya sp.* by determining the possible number and pattern of developmental modules defining the shape of the hind wings. Different hypotheses were formulated and tested using MINT software (Modularity and Integration Tool, ver 1.5) as to possible developmental boundaries based on wing venation. A total of 180 points were used to trace and outline the margins as well as the major of the hind wings. Results of this study show that wing compartments bounded by major veins are potential candidates for separate developmental modules that may correspond to distinct cell lineages and domains of gene expression. The entire hind wing was observed to have 3 best-fit models indicating that the compartments could be considered as autonomous units of morphological variation that may correspond to domains of gene expression. Major veins serve not only as boundaries but also as active center of integration.

Keywords: hind wing, integration, modularity, modules, *Oxya sp.*

Introduction

Insect wings have large contributions to their unparalleled success. The wings bring superiority and competence in the field of foraging, calling, finding places for spawning and avoiding predators¹. Insect wings are divided into compartments by complex network of netted veins reflecting their roles in giving support during flight. It has been hypothesized that subdivisions or compartments of insect wings correspond to a distinct cell lineages and domains of gene expression^{2,3}. Each wing compartment is a potential candidate of being separate and distinct developmental module that is reflected in phenotypic and genetic variation². Hence, concept of modularity comes into mind. Modularity is related to the concept of “morphogenetic fields”⁴ for they are constituted by the localized developmental processes that take place within them, and to the concept of “morphological integration” as such modules are structural units that are internally integrated by developmental interactions^{5,6}. Thus, modularity confers a degree of evolutionary autonomy to the sets of traits integrating a module by allowing selection to optimize individual parts without interfering with others⁷.

However, the question whether the entire hind wing is a single module or whether the compartments, even the smaller parts could be considered as autonomous units of morphological variation that may correspond to domains of gene expression, still remained ambiguous. In this study, Modularity and Integration (MINT) analysis tool^{1,3,7} was used to determine the autonomous unit of morphological variation that could be considered as developmental modules. The approach of this

study was that modules are considered as subsets of dimensions embedded in phenotypic space. This allows traits to be integrated into more than one module and suggests a natural approach for testing a priori hypotheses of modularity by fitting competing hypotheses to observed covariance matrices, searching for the best- supported causal explanation⁷. Hence, the objective of this study is to determine the possible number and pattern of developmental modules defining the hind wings of *Oxya sp* (figure 1). This study aims to delimit the spatial domain of developmental modules in the hind wings and to determine whether the compartments, even smaller parts of the wings, could be considered as “autonomous unit of morphological variation”.

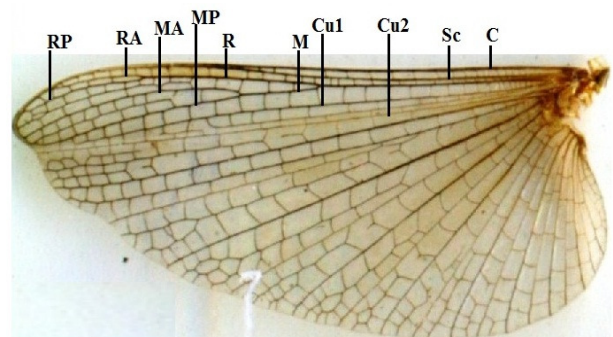


Figure- 1

Hind wing of *Oxya sp*, showing veins that may serve as boundaries of the hypothesized developmental modules. Legend: C= Costa, Sc= Subcosta, R= Radius, RA= Radius Anterior, RP= Radius Posterior, M=Media, MA=Media Anterior, MP=Media Posterior, Cu= Cubitus 1 and 2

Material and Methods

Detached hindwings were mounted in a clear and clean slides properly labelled. Digital images of the hind wings were acquired and were obtained using a Hewlett-Packard Jacket 2400 Scanner at 1200 dots per inch (dpi) resolution. Images were cropped, labelled and saved one by one. Through TPSdig2 ver 2.12⁸, a total of 195 points were used for outlining the shape as well as the wing venation pattern of the hind wings. After outlining, the TPS curve was then converted into landmarks points (XY) using TPSutil ver 1.44⁹ which served as the raw data for the analysis.

Modularity and integration is concern with the degree of covariation between parts of a structure¹⁰, which can be studied by means of morphometric methods. In this study, different hypothesis (table - 1) were formulated and tested to determine the possible number and pattern of developmental modules and boundaries defining the hind wings.

Modularity and Integration Tool (MINT) for morphometric Data⁷ was used to study modularity and integration in grasshopper's hindwings. The software calculates the matrix correlations between expected and observed covariance matrices. The data sets were loaded, and then a set of models were built and loaded. A total of 12 *a priori* models for the hindwings (figure - 2) were constructed with the help of the model building tool option of the software. MINT assumes that the data themselves have modular structure, and by partitioning the entire data space into orthogonal subspaces, covariance matrices were then computed based on the modified data structures⁷.

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. The lower γ^* value imply will high degree of similarity between the observed data and the proposed model. Meanwhile, a low P-value (<0.05) corresponds to large values of γ^* value, which implies a large difference between data and the model and thus a poor fit model⁷.

Results and Discussion

Table - 2 shows the gamma (γ) values and p-values computed for each model in the hindwing. The result only shows the top three best fit models for the hindwing. Results show the hind wing have slight difference in the ranks of the best fit models as supported by their yielded P-value and lowest gamma (γ^*) value. However, consistency of the models in both sexes and between left and right hind wings is evident (table - 2, figure - 3).

Results in this study is in conformity with a number of studies suggesting that insect wings, including small part of the wings, are being partitioned into compartments, that these modules serves as autonomous unit of morphological variation and each

of compartments is a separate developmental module^{5,11-14}. These compartments may have sets of genes controlling them during wing development^{2,11,15,13,14}. Looking at the hindwings, veins play a crucial role in delimiting the spatial domain of each module especially the major veins serving as possible boundaries. The compartment boundaries does not only serve as a delimiter between modules but also served as active center of integration and an origin of morphogen gradients from which crucial patterning of wing vein signal originate^{12,16,17,18}. These signals initiate regulatory interactions that subdivide the wings into series of sectors with discrete boundaries^{1,17,19,20}.

Conclusion

Results of this study show that wing compartments bounded by major veins are potential candidates for separate developmental modules that may correspond to distinct cell lineages and domains of gene expression. The entire hind wing was observed to have 3 best-fit models indicating that the compartments, even the smaller parts of the wings, could be considered as autonomous units of morphological variation that may correspond to domains of gene expression. Each compartment is considered separate different developmental module representing individual units of selection that are subjected to different genetic control. Major veins serve not only as boundaries but also as active center of integration.

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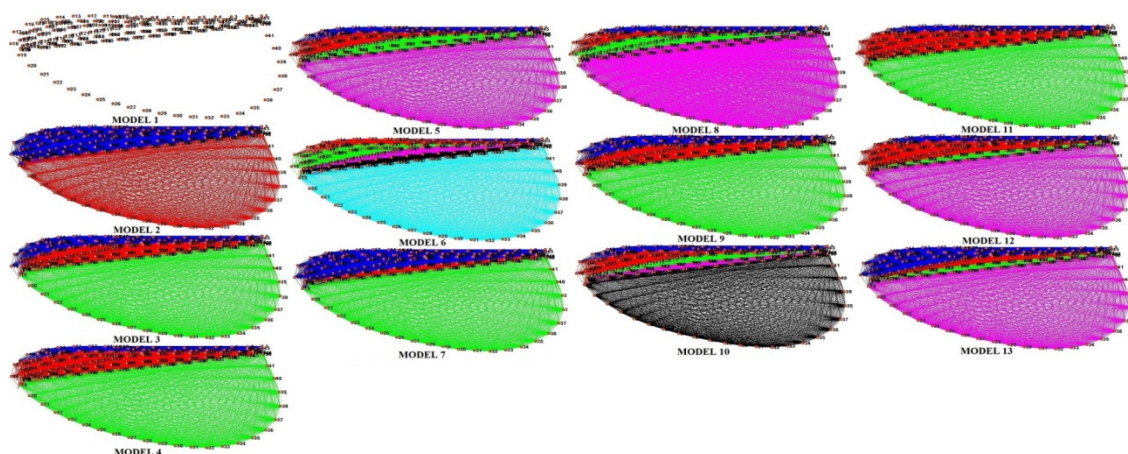


Figure- 2
 Models used in this study for the hind wings of *Oxya sp.*

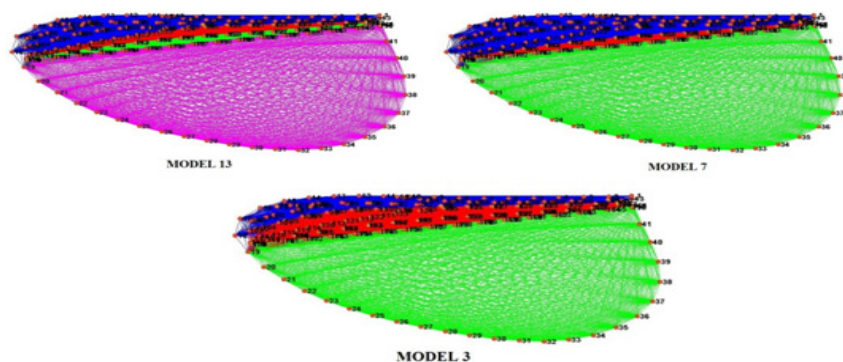


Figure – 3
 Best fit model for the hind wing of both sexes of *Oxya sp.*

Table – 1
A priori developmental modules of modularity tested in this study. Modules correspond to regions of the hind wing of Rice grasshoppers, *Oxya sp* as hypothesized

Models	Modules	Description
H ₀	0	Null model, predicting absence of modular structure
H ₁	2	First module is bounded by Costa (C) and Cubitus 2 (Cu2)
		Second module is bounded by Cubitus 2 (Cu2) and Posterior margin of the wings
H ₂	3	First module is bounded by Costa (C) and Media posterior (MP)
		Second module is bounded by Media posterior (MP) and Cubitus 2 (Cu2)
		Third module is bounded by Cubitus 2 (Cu2) and Posterior margin of the wings
H ₃	3	First module is bounded by Costa (C) and Radius posterior (RP)
		Second module is bounded by Radius posterior (RP) and Cubitus 2 (Cu2)
		Third module is bounded by Cubitus 2 (Cu2) and Posterior margin of the wings
H ₄	4	First module is bounded by Costa (C) and Radius posterior (RP)
		Second module is bounded by Radius posterior (RP) and Media posterior (MP)
		Third module is bounded by Media posterior (MP) and Cubitus 2 (Cu2)
		Fourth module is bounded by Cubitus 2 (Cu2) and Posterior margin of the wings
H ₅	6	First module is bounded by Costa (C) and Subcosta (Sc)
		Second module is bounded by Subcosta (Sc) and Radius posterior (RP)
		Third module is bounded by Radius posterior (RP) and Media posterior (MP)
		Fourth module is bounded by Media posterior (MP) and Cubitus 1 (Cu1)
		Fifth module is bounded by Cubitus 1 (Cu1) and Cubitus 2 (Cu2)
		Sixth module is bounded by Cubitus 2 (Cu2) and Posterior margin of the wings
H ₆	3	First module is bounded by Costa (C) and Cubitus 1 (Cu1)
		Second module is bounded by Cubitus 1 (Cu1) and Cubitus 2 (Cu2)
		Third module is bounded by Cubitus 2 (Cu2) and Posterior margin of the wings
H ₇	4	First module is bounded by Costa (C) and Radius anterior (RA)
		Second module is bounded by Radius anterior (RA) and Media anterior (MA)
		Third module is bounded by Media anterior (MA) and Cubitus 1 (Cu1)
		Fourth module is bounded by Cubitus 1 (Cu1) and Posterior margin of the wings.
H ₈	3	First module is bounded by Costa (C) and Media anterior (MA)
		Second module is bounded by Media anterior (MA) and Cubitus 2 (Cu2)
		Third module is bounded by Cubitus 2 (Cu2) and Posterior margin of the wings
H ₉	5	First module is bounded by Costa (C) and Radius posterior (RP)
		Second module is bounded by Radius posterior (RP) and Media posterior (MP)
		Third module is bounded by Media posterior (MP) and Cubitus 1 (Cu1)
		Fourth module is bounded by Cubitus 1 (Cu1) and Cubitus 2 (Cu2)
		Fifth module is bounded by Cubitus 2 (Cu2) and Posterior margin of the wings
H ₁₀	3	First module is bounded by Costa (C) and Radius anterior (RA)
		Second module is bounded by Radius anterior (RA) and Cubitus 2 (Cu2)
		Third module is bounded by Cubitus 2 (Cu2) and Posterior margin of the wings
H ₁₁	4	First module is bounded by Costa (C) and Radius anterior (RA)
		Second module is bounded by Radius anterior (RA) and Cubitus 1 (Cu1)
		Third module is bounded by Cubitus 1 (Cu1) and Cubitus 2 (Cu2)
		Fourth module is bounded by Cubitus 2 (Cu2) and Posterior margin of the wings
H ₁₂	4	First module is bounded by Costa (C) and Media posterior (MP)
		Second module is bounded by Media posterior (MP) and Cubitus 1 (Cu1)
		Third module is bounded by Cubitus 1 (Cu1) and Cubitus 2 (Cu2)
		Fourth module is bounded by Cubitus 2 (Cu2) and Posterior margin of the wings

Table – 2
Computed γ - value and P-value for the left and right hindwings of male Rice grasshoppers, *Oxya sp.* Only the top three (3) best fit models are tabulated

Sex	Wing	Rank	Model	γ - VALUE	P-VALUE
Male	Left	1	13	0.11383	1
		2	3	0.12833	1
		3	7	0.1315	1
	Right	1	13	0.067939	1
		2	7	0.08246	1
		3	3	0.095515	1
Female	Left	1	13	0.1138	1
		2	3	0.1283	1
		3	7	0.1315	1
	Right	1	13	0.0623	1
		2	3	0.0694	1
		3	7	0.0833	1