

Describing Compartmentalization in the Fore-wing of Populations of the Rice Grasshoppers, *Oxya* sp. (Acrididae: Orthoptera)

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

Department of Biological Sciences, College of Science and Mathematics,
Mindanao State University-Iligan Institute of Technology,
9200 Iligan City, Philippines

*For Correspondence: kimverly_hazel@yahoo.com

ABSTRACT

The fore-wings of Rice grasshoppers, *Oxya* sp. are divided into compartments by a complex network of veins. There has been a long term hypothesis suggesting that wing compartments bounded by veins may correspond to distinct cell lineages and domains of gene expression. Hence, this study was conducted to determine the patterns of modularity and integration in the fore-wings of the Rice grasshoppers, *Oxya* sp. A total of 180 points were used to outline the margins of the fore-wings. Results showed that the fore-wing of *Oxya* sp. is partitioned into 6 developmental modules. The consistency in the number and pattern of developmental modules in the fore-wings suggests that the wings are highly conserved indicating genetic conservatism in the morphological spaces. Results also suggest that compartments are considered an autonomous unit of gene regulation and major veins served as boundaries and as active center of integration.

Keywords: Fore-wings, Modules, Modularity and Integration, *Oxya* sp.

INTRODUCTION

Wing is an essential part in the daily life of an insect and is considered to be an important organ. Wing traits evolve rapidly to respond to various environmental conditions (Cui and Liu, 2001) and therefore, have large contribution to the unparallel success and wide diversity of insects. Since wing venation is species-specific it is also used taxonomically (Francoy *et al.*, 2009). Several studies on compartmentalization in insect wings suggest that one or set of genes control wing development (Garcia-Bellido and de Celis, 1992; Sturtevant and Bier, 1995; Gilbert *et al.*, 1996; Biehs *et al.*, 1998; Torres *et al.*, 2010). Wing compartments may respond to distinct cell lineages and domains of gene expression (Lawrence, 1992; Klingenberg *et al.*, 2002; Demayo *et al.*, 2011; Tabugo *et al.*, 2011), thus are promising candidates for being separate developmental modules.

Modularity has recently become an active area for investigation in evolutionary developmental biology and other related discipline (Klingenberg, 2008). This describes the general idea that biological systems at many hierarchal levels are about the differences in the degree of integration of part within and between sets of traits (Klingenberg, 2008). It is related to the concept of "morphogenetic field" (Gilbert *et al.*, 1996; Klingenberg *et al.*, 2001; Wilkins, 2002) as they are constituted by localized developmental processes that takes place within them; herewith morphological modules have concrete spatial dimensions (Klingenberg *et al.*, 2002). Since modules are structural units that are internally integrated by developmental interactions (Klingenberg *et al.*, 2002; Demayo *et al.*, 2011) and modularity and integration are

concerned with the degree of covariation between parts of a structure, these can be studied by means of morphometric methods (Klingenberg, 2009). The question however is whether the entire wing is a single module or whether the compartments, even the smaller parts of the wings, could be considered as autonomous units of morphological variation. The issue that may correspond to domains of gene expression, still remained ambiguous, thus this study was conducted. The investigation focused on *Oxya* sp.

Various studies have been conducted looking into the wings of other insects such as fruit fly, bees and dragonfly (Klingenberg *et al.*, 2001; Klingenberg, 2009; Tabugo *et al.*, 2011). However, there are no studies yet looking into the wings of grasshoppers. Grasshopper wings provide many morphological landmarks bounded by veins thus are potential candidates of developmental modules.

In this study, *Oxya* sp. grasshoppers were collected at three provinces of Mindanao: Lanao del Norte, Misamis Occidental and Zamboanga del Sur. The morphometric data in the fore-wings were used for studying modularity and integration. Modularity and Integration (MINT) analysis tool (Marquez, 2008a) was used to determine the autonomous unit of morphological variation that could be considered as developmental modules. Morphological approach in identifying developmental modules is not different from the approach based on genetic interaction, thus the objectives of this study are to determine the possible number and pattern of developmental modules defining the fore-wings of *Oxya* sp. using the Modularity and Integration Analysis Tool (MINT ver 1.5, Marquez, 2008b). This study aims to delimit the spatial domain of developmental modules in the fore-wings of Rice grasshoppers, *Oxya* sp. and to determine whether such pattern of developmental modules between populations of *Oxya* sp. holds true even for different geographical areas. This study also aims to determine whether the compartments, even smaller parts of the wings, could be considered as “autonomous unit of morphological variation”.

Understanding modules in the wings of the grasshoppers can be informative about the underlying biological process of compartmentalization in the wings. Likewise, understanding covariation between modules can have substantial implications for understanding genetic variation and the potential of the species for evolutionary change (Klingenberg, 2008).

MATERIAL AND METHODS

Samples were collected at selected provinces in Mindanao namely: Lanao del Norte, Misamis Occidental and Zamboanga del Sur by using sweep nets and were placed in a properly labelled container filled with 70% ethanol. Through visual inspection of genitalia the sex of samples were identified.

The fore-wings were detached and were mounted neatly on clean and clear slides and were properly labelled. Mounted slides were scanned using a Hewlett-Packard Jacket 2400 Scanner with 1200 dots per inch (dpi). Images were cropped, labelled and saved one by one. Through TPSdig2 (ver 2.12, Rohlf, 2008), a total of 180 points were used for outlining the shape, as well as the wing venation pattern of the fore-wings of *Oxya* sp. (Fig. 1). After outlining, the TPS curve was then converted into landmarks points (XY) using TPSutil (Rohlf, 2009) which served as the raw data for the analysis.

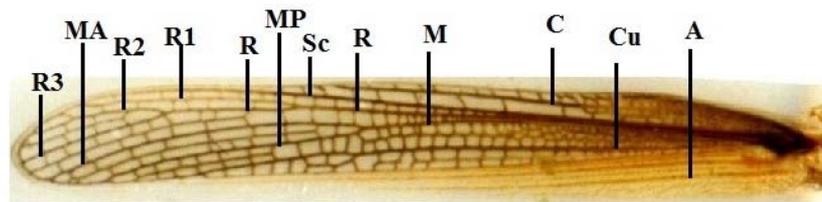


Fig. 1: Rice grasshoppers, *Oxya* sp., fore-wings showing veins that may serve as boundaries of the hypothesized developmental modules. Legend: C = Costa, Sc = Subcosta, R = Radius, M = Media, Cu = Cubitus and A = Anal vein.

Modularity and Integration Tool (MINT) for Morphometric Data version 1.5 (Marquez, 2008b) was used to study modularity and integration in the fore-wings of the selected population of *Oxya* sp. 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 11 *a priori* models for the fore-wings (Table 1, Fig. 2) *Oxya* sp. were constructed with the help of the model building tool option of the software. MINT assumes that the data themselves have modular structure, and that by partitioning the entire data space into orthogonal subspaces, covariance matrices were then computed based on the modified data structures (Marquez, 2008a).

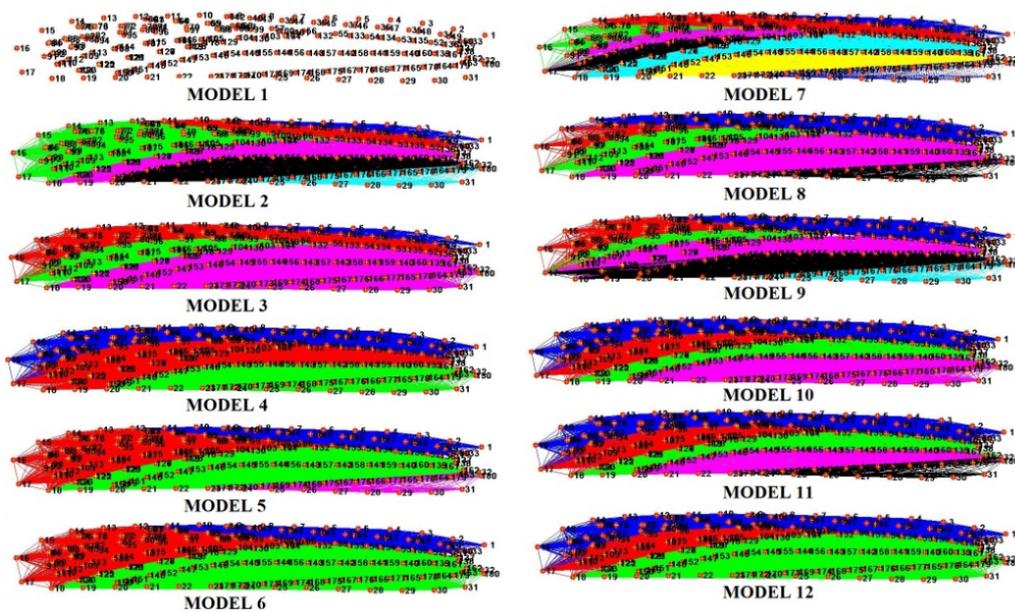


Fig. 2: Models used in this study for the fore-wings of Rice grasshoppers, *Oxya* sp.

The patterns of variational modularity were tested using Gamma (γ^*) 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 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 model (Marquez, 2008a).

Table 1. A priori developmental modules of modularity tested in this study. Modules correspond to regions of the fore-wing of *Oxya* sp. as hypothesized.

MODELS	MODULES	DESCRIPTION
M1:H ₀	0	Null model, predicting absence of modular structure First module is bounded by anterior margin and Costa Second module is bounded by Costa (C) and Subcosta (Sc) Third module is bounded by Subcosta (Sc) and Media posterior (MP) Fourth module is bounded by Media posterior (MP) and Cubitus (Cu) Fifth module is Bounded by Cubitus (Cu) and Anal Vein (A) Sixth module is bounded by Anal vein (A) and Posterior margin of the wings.
M2:H ₁	6	First module is bounded by anterior margin and Costa Second module is bounded by Costa (C) and Radius (R3) Third module is bounded by Radius (R3) and Media posterior (MP) Fourth module is bounded by Media posterior (MP) and Posterior margin of the wings.
M3:H ₂	4	First module is bounded by anterior margin and Subcosta (Sc) Second module is bounded by Subcosta (Sc) and Media posterior (MP) Third module is bounded by Media posterior (MP) and Anal Vein (A) Fourth module is bounded by Anal vein (A) and Posterior margin of the wings.
M4:H ₃	3	First module is bounded by anterior margin and Costa (C) Second module is bounded by Costa (C) and Radius (R3) Third module is bounded by Radius (R3) and Cubitus (Cu) Fourth module is bounded by Cubitus (Cu) and posterior margin of the wings.
M5:H ₄	4	First module is bounded by anterior margin and Subcosta (Sc) Second module is bounded by Subcosta (Sc) and Media posterior (MP) Third module is bounded by Media posterior (MP) and Anal Vein (A) Fourth module is bounded by Anal vein (A) and Posterior margin of the wings.
M6:H ₅	3	First module is bounded by anterior margin and Subcosta (Sc) Second module is bounded by Subcosta (Sc) and Media posterior (MP) Third module is bounded by Media posterior (MP) and Posterior margin of the wings.
M7:H ₆	8	First module is bounded by anterior margin and Costa Second module is bounded by Costa (C) and Radius (R1) Third module is bounded by Radius (R1 and R3) Fourth module is bounded by Radius (R3) and Media anterior (MA) Fifth module is bounded by Media anterior (MA) and Media posterior (MP) Sixth module is bounded by Media posterior (MP) and Cubitus (Cu) Seventh Module is bounded by Cubitus (Cu) and Anal vein (A) Eighth module is bounded by Anal vein (A) and Posterior margin of the wings.
M8:H ₇	5	First module is bounded by the anterior margin of the wings and Radius (R1) Second module is bounded by Radius (R1 and R3) Third module is bounded by Radius (R3) and Media posterior (MP) Fourth module is bounded by Media posterior (MP) and Anal Vein (A) Fifth module is bounded by Anal vein (A) and Posterior margin of the wings.
M9:H ₈	6	First module is bounded by anterior margin and Subcosta (Sc) Second module is bounded by Subcosta (Sc) and Radius (R3) Third module is bounded by Radius (R3) and Media anterior (MA) Fourth module is bounded by media anterior (MA) and Cubitus (Cu) Fifth module is bounded by Cubitus (Cu) and Anal vein (A) Sixth module is bounded by Anal vein (A) and Posterior margin of the wings.
M10:H ₉	4	First module is bounded by the anterior margin of the wings and Radius (R3) Second module is bounded by Radius (R3) and Media posterior (MP) Third module is bounded by Media posterior (MP) and Cubitus (Cu) Fourth module is bounded by Cubitus (Cu) and posterior margin of the wings.
M11:H ₁₀	5	First module is bounded by the anterior margin of the wings and Radius (R3) Second module is bounded by Radius (R3) and Media posterior (MP) Third module is bounded by Media posterior (MP) and Cubitus (Cu) Fourth module is bounded by Cubitus (Cu) and Anal vein (A) Fifth module is bounded by Anal vein (A) and Posterior margin of the wings.
M12:H ₁₁	3	First module is bounded by the anterior margin of the wings and Radius (R3) Second module is bounded by Radius (R3) and Media posterior (MP) Third module is bounded by Media posterior (MP) and Posterior margin of the wings.

RESULTS AND DISCUSSION

Table (2) shows the gamma (γ^*) and p-values computed for each model in the fore-wing of *Oxya* sp. Results show that Model 2 yielded a P-value of 1 and a lowest gamma (γ^*) value, indicating that the proposed model and observed data are not significantly different. Model 2 therefore qualify as the best fit model and was fairly consistent best-fit model for the fore-wings for both male and female *Oxya* sp. (Fig. 3). In this model, the fore-wing is partitioned into six (6) developmental modules: (1) Module 1 is bounded by anterior margin and Costa (C), (2) second module is bounded by Costa (C) and Subcosta (Sc), (3) Third module is bounded by Subcosta (Sc) and Media posterior (MP), (4) fourth module bounded by Media posterior (MP) and

Cubitus (Cu), (5) fifth module is bounded by Cubitus (cu) and Anal vein (A), and (6) sixth module is bounded by anal vein and posterior margin of the fore-wing.



Fig. 3: Best fit model for grasshopper for both male and female of Rice grasshoppers, *Oxya* sp. in all sites which defines the shape of the fore-wing.

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

Sex	Wing	Location	Rank	Model	γ -VALUE	P-VALUE
Male	Left	Lanao del Norte	1	2	0.17502	1
			2	5	0.22753	1
			3	4	0.24128	1
		Misamis Occidental	1	2	0.20544	1
			2	5	0.30142	1
			3	6	0.3212	1
	Zamboanga del Sur	1	2	0.185	1	
		2	5	0.22216	1	
		3	6	0.23745	1	
	Right	Lanao del Norte	1	2	0.19177	1
			2	5	0.27841	1
			3	6	0.297	1
Misamis Occidental		1	2	0.19902	1	
		2	5	0.2853	1	
		3	8	0.28852	1	
Zamboanga del Sur	1	2	0.10792	1		
	2	5	0.14926	1		
	3	6	0.16433	1		
Female	Left	Lanao del Norte	1	2	0.19177	1
			2	5	0.27841	1
			3	6	0.297	1
		Misamis Occidental	1	2	0.14016	1
			2	5	0.24745	1
			3	4	0.26292	1
	Zamboanga del Sur	1	2	0.15437	1	
		2	5	0.21894	1	
		3	6	0.2363	1	
	Right	Lanao del Norte	1	2	0.30036	1
			2	5	0.33391	1
			3	6	0.3506	1
Misamis Occidental		1	2	0.33107	1	
		2	5	0.33943	1	
		3	6	0.35055	1	
Zamboanga del Sur	1	2	0.10792	1		
	2	5	0.14926	1		
	3	6	0.16433	1		

The consistency of Model 2 as the best fit model of the fore-wings of *Oxya* sp. in all geographical location is evident. This indicates that the modularity in the fore-wings is highly conserved and there is genetic conservatism in the morphological spaces in the fore-wing. Consistency of the best fit model suggests that there is not much variation in the spatial arrangement of developmental modules in the fore-wings of *Oxya* sp. Each fore-wing appears to be internally coherent developmental processes that produce shape variation thus the wings can be considered as single developmental module (Klingenberg *et al.*, 2001) occupying a specific morphological domain and corresponds to a single morphogenetic field (Gilbert *et al.*, 1996). The different modules can evolve independently, at least to some extent, without disrupting

functions at the level of whole organism (Klingenberg, 2005; Klingenberg, 2008; Torres *et al.*, 2010; Tabugo *et al.*, 2011).

However, the results of this study confirmed a number of studies suggesting that insect wings are being partitioned into compartments, and that these modules serve as autonomous units of morphological variation and each compartment is a separate developmental module (Cowley and Atchley, 1990; Cavicchi *et al.*, 1991; Zimmerman *et al.*, 2000; Klingenberg *et al.*, 2001; Torres *et al.*, 2010; Tabugo *et al.*, 2011). Results show that main veins serve as boundaries for each developmental module. Compartment boundaries (veins) not only serve as a delimiter between autonomous unit of developmental domains or modules but also serves as active center of integration from which crucial patterning signal originate (Lawrence and Struhl, 1996; Milan and Cohen, 2000; Klingenberg and Zaklan, 2000; Tabugo *et al.*, 2011). These signals initiate regulatory interactions that subdivide the wings into series of sectors with discrete boundaries (Sturtevant and Bier, 1995; Lawrence and Struhl, 1996; Sturtevant *et al.*, 1997; Biehs *et al.*, 1998; Milan and Cohen, 2000).

CONCLUSION

Results showed that in all provinces, the fore-wings of the Rice grasshoppers, *Oxya* sp for both male and female and between left and right, are partitioned into 6 developmental modules: (1) Module 1 is bounded by anterior margin and Costa (C), (2) second module is bounded by Costa (C) and Subcosta (Sc), (3) Third module is bounded by Subcosta (Sc) and Media posterior (MP), (4) fourth module bounded by Media posterior (MP) and Cubitus (Cu), (5) fifth module is bounded by Cubitus (Cu) and Anal vein (A), and (6) sixth module is bounded by anal vein (A) and posterior margin of the fore-wing.

Fair consistency in the number and patterns of the hypothesized developmental modules in the fore-wings imply that the wings of *Oxya* sp. are highly conserved indicating genetic conservatism.

ACKNOWLEDGEMENT

The authors would like to thank Sir Muhmin Michael E. Manting and Prof. Sharon Rose M. Tabugo for thier support and insights towards the study. To DOST-SEI (ASTHRDP) for the scholarship grant.

REFERENCES

- Biehs, B., M.A. Sturtevant and E. Bier, (1998). Boundaries in the *Drosophila* wing imaginal disc organize vein-specific genetic programs. *Development*, 125: 4245–4257.
- Cavicchi, S., G. Giorgi, V. Natali and D. Guerra. (1991). Temperature-related divergence in experimental populations of *Drosophila melanogaster*. III. Fourier and centroid analysis of wing shape and relationship between shape variation and fitness. *J. Evol. Biol.*, 4: 141–159.
- Cowley, D.E. and W.R. Atchley (1990). Development and quantitative genetics of correlation structure among body parts of *Drosophila melanogaster*. *American Nat.*, 135: 242–268.
- Cui, B. and G. Liu. (2011). Research Significant of Wing and Vein among Insecta. *International Journal of Biology*, 3: 80-183.

- Demayo, C.G, R.B. Rampola and M.A.J. Torres. (2011). Describing Developmental Modules in the Fore- and Hindwings of the Nymphalid Butterfly, *Ypthima lisandra* using Modularity and Integration Software. *Aust. J. Basic and Appl. Sci.*, (6): 733-738.
- Francoy, T.M., R.A.O. Silva, P. Nunes-Silva, C. Menezes and V.L. Imperatriz-Fonseca. (2009). Gender identification of five genera of stingless bees (Apidae, Meliponini) based on wing morphology. *Genetics and Molecular Research* 8(1): 207-214.
- Garcia-Bellido, A. and J.F. De Celis, (1992). Developmental Genetics of the venation patterns of *Drosophila*. *Annu. Rev. Genet.*, 26: 277–304.
- Gilbert, S.F., J.M. Opitz and R.A. Raff, (1996). Resynthesizing evolutionary and developmental biology. *Dev. Biol.*, 173: 357–372.
- Klingenberg, C.P. (2005). Developmental constraints, modules and evolvability, Pages 219–247 in B. Hallgrímsson and B.K. Hall, eds. *Variation*. San Diego, Academic Press.
- Klingenberg, C.P. (2008). Morphological integration and developmental modularity. *Annual. Rev. ecol. vol. Syst.* 39(1):115–132, doi: 10.1146/annurev.ecolsys.37.091305.110054.
- Klingenberg, C.P. (2009). Morphometric integration and modularity in configurations of landmarks: Tools for evaluating a-priori hypotheses. *Evolution & Development*, 11: 405–421.
- Klingenberg, C.P. (2010). Evolution and development of shape: integrating quantitative approaches. *Nature Reviews Genetics*, 11: 623–635.
- Klingenberg, C.P., A.V. Badyaev, S.M. Sowry, and N.J. Beckwith (2001). Inferring developmental modularity from morphological integration: analysis of individual variation and asymmetry in bumblebee wings. *Am. Nat.*, 157: 11–23.
- Klingenberg, C.P., M. Barluenga and A. Meyer (2002). Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. *Evolution* 56: 1909-1920.
- Klingenberg, C.P. and S.D. Zaklan. (2000). Morphological integration between developmental compartments in the *Drosophila* wing. *Evolution*, 54: 1273–1285.
- Lawrence, P.A. (1992). *The Making of a Fly: the Genetics of Animal Design*. Blackwell scientific, Oxford, UK.
- Lawrence, P.A. and Struhl, G. (1996). Morphogens, compartments, and pattern: Lessons from *Drosophila*? *Cell*, 85(7): 951-961.
- Marquez, E J. (2008a). A statistical framework for testing modularity in multidimensional data. *Evolution*, 62: 2688–2708.
- Marquez, E.J. (2008b). *MINT: Modularity and Integration Analysis tool for Morphometric Data*. Version 1.0 b (compiled 09/07/08). Mammals Division, University of Michigan Museum of Zoology.
- Milán M. and E. Cohen (2000). Subdividing cell populations in the developing limbs of *Drosophila*: do wing veins and leg segments define units of growth control? *Dev. Biol.*, 217: 1–9.
- Rohlf, J.F. (2008). TPSDig version 2.12. Department of Ecology and Evolution, State University of New York at Stony Brook, New York.
- Rohlf, J.F. (2009). TPSUtil version 1.44. Department of Ecology and Evolution, State University of New York at Stony Brook, New York.
- Sturtevant, M.A. and E. Bier (1995). Analysis of the genetic hierarchy guiding wing vein development in *Drosophila*. *Development*, 121: 785–801.

- Sturtevant, M.A., B. Biehs, E. Marin and E. Bier. (1997). The *spalt* gene links the A/P compartment boundary to a linear adult structure in the *Drosophila* wing. *Development*, 124: 21–32.
- Tabugo, S.R., M.A. Torres and C.G. Demayo (2011). Determination of Developmental Modules and Conservatism in the Fore- and Hindwings of two Species of Dragonflies, *Orthetrum sabina* and *Neurothemis ramburii*. *Int. J. Agric. Biol.*, 13: 541-546.
- Torres, M.A.J., L.A. Adamat, M.M.E. Manting, S.R.M. Tabugo, R.C. Joshi, L. Sebastian, A.T. Barrion and C.G. Demayo (2010). Developmental modules defining the shape of the forewing of *Scotinophara coarctata*. *Egypt. Acad. J. biolog. Sci.*, 3(1): 105-112.
- West-Eberhard, M.J. (2003). *Developmental plasticity and evolution*. New York: Oxford University Press.
- Wilkins, A.S. (2002). *The evolution of developmental pathways*. Sinauer Associates, Sunderland, MA.
- Zimmerman, E.; Palsson, A. and Gibson, G. (2000). Quantitative trait loci affecting components of wing shape in *Drosophila melanogaster*. *Genetics*, 155: 671–683.