



## Describing Modularity in the Forewings of the Mango Leafhopper (*Idioscopus clypealis*)

Mark Ronald S. Manseguiao\*, Elbridge D. Bonachita, Liza A. Adamat and Cesar G. Demayo

Department of Biological Sciences, MSU-Iligan Institute of Technology, Iligan, Philippines

\*Corresponding author: [mark.manseguiao@gmail.com](mailto:mark.manseguiao@gmail.com)

**Abstract.** The effects of environmental factors on genetic modules is important in understanding the development of organisms. This study was conducted to determine the developmental modules in the forewings of the mango leafhopper (*Idioscopus clypealis*) and establish if variation exists between populations attacking different hosts and between sexes. A total of 199 landmarks points were used to outline the margins and wing venation of the leafhopper. Models were constructed to hypothesize modularity in the wings. The  $\gamma^*$  (Gamma\*) test for Goodness of fit (GoF) was used to assess the best fit model. Results showed that the forewings of *I. clypealis* is partitioned into 5 developmental modules bounded by the major veins in all populations except for the left forewing of the female leafhopper in Paho variety ( $\gamma^* = -0.25572$ ,  $P = 1$ ). The results suggest that wing development is conserved but may be affected by host.

**Keywords.** *Idioscopus clypealis*; Gamma\*

MSC. 92C37

**Received:** November 2, 2016

**Accepted:** March 23, 2017

Copyright © 2017 Mark Ronald S. Manseguiao, Elbridge D. Bonachita, Liza A. Adamat and Cesar G. Demayo. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

### 1. Introduction

Organisms have an inherently modular body plan integrated to form complex structures that serve a whole [1]. Understanding modular organization is important to appreciate the plasticity of form and in the development of the individual [2]. Environmental factors acting on genetic modules come to affect the phenotype inducing variability which may confer reproductive

success. A modular body plan is clearly seen in insects. Insect wings are found to be controlled by one or a set of genes and is often used for insect taxonomy [3, 4]. Moreover, insect wings evolve rapidly which can easily be measured using phenotypic methods [5]. Morphometric methods measure these phenotypic changes which allows us to define the changes occurring in a population and gauge adaptation.

In this study, morphometric methods are employed to identify the developmental modules in the wing of the mango leafhopper (*Idioscopus clypealis*). This study further investigates if sexual dimorphism occurs in developmental modules and if hosts confers variability in these developmental modules.

In the Philippines, the leafhopper (*I. clypealis*) is a major pest of mangoes (*Mangifera indica*). Despite pesticide regimes, infestation persists as flowering is induced to increase harvest affecting harvest yield aside from the typhoons that visit the country every year [6–8]. The leafhopper extracts the sap from inflorescences causing drying and dropping of the flowers [9].

Understanding the variation in developmental modules is informative of host effects on genetic modules in the mango leafhopper. Moreover, potential for evolutionary change is assessed to better understand genetic variation in the species studied.

## 2. Materials and Methods

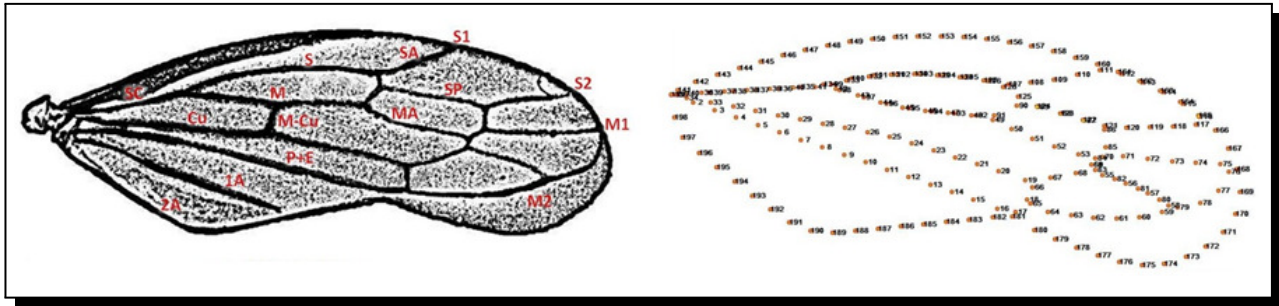
### Collection and Processing of Samples

Samples of *I. clypealis* were collected from tress ofsoursop (*Annona muricata* L.) and from two varieties of mango (*Mangifera indica* L.) specifically the Cebu variety and Paho variety. Collection of the specimens were done by carefully encasing whole leaf branches in a clear plastic bag with cotton balls dipped in 100% ethanol. After a 2-3 minutes the plastic bag would be carefully removed taking care that leafhoppers remained trapped. The bag would then be shaken to collect the leafhoppers at the bottom of the bag and then transferred to a container filled with 100% ethanol. The collected samples were then separated by host and by sex.

The forewings of the leafhoppers were detached and mounted in a clear glass slides with a small droplet of glycerine and properly labelled. Wing images were taken using a stereomicroscope with camera attachment.

### Model Construction and Model Testing

Images were outlined using 199 points to outline the shape and the wing venation pattern of *I. clypealis* as shown in Figure 1 using tpsDig software version 2.17 [10]. The outlines were then converted into two dimensional landmark points using tpsUtil software [11]. The landmark points were then loaded to the MINT (Modularity and Integration Analysis Tool) software version 1.61 [12].

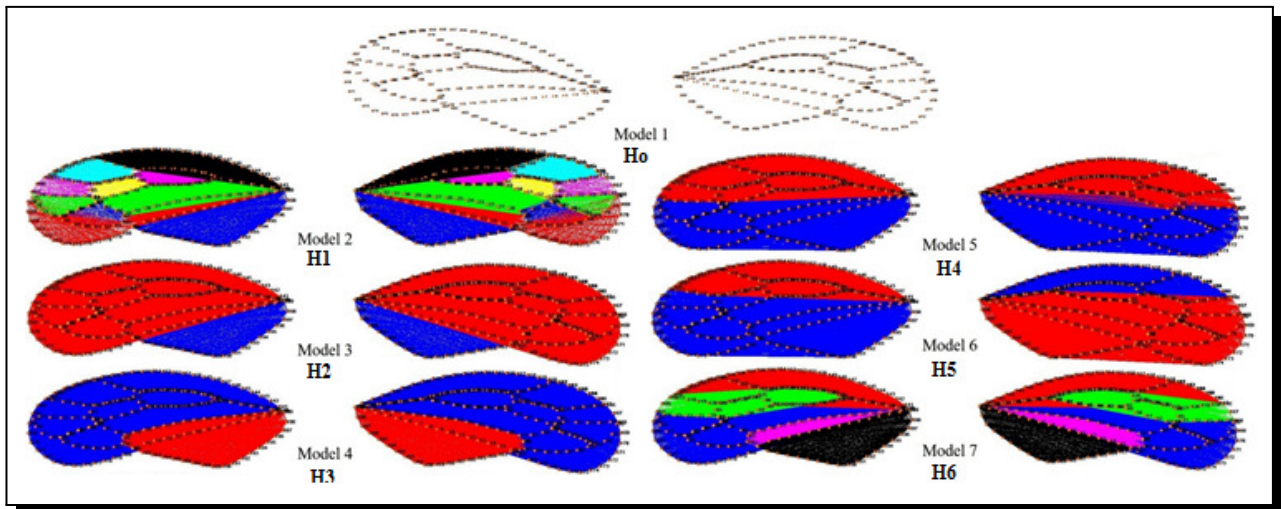


**Figure 1.** Image (left) and outline of landmark points (right) of the forewing of *I. clypealis*.

The leafhopper wings have five main vein stems as is observed in Hemiptera wings: Costa (C), Subcosta (Sc), Media (M), Plicus and Empusal (P+E), Cubitus (Cu) and Anal (A) veins (Figure 2). Using the points as bounded by the veins, seven hypotheses (Figure 2, Table 1) were constructed to determine if there were host variations in wing modularity and if differences exist between the left and the right forewings and by gender.

**Table 1.** The veins in the forewing that possibly defines the boundaries of the developmental modules in the hypothesized models.

Models	Modules	Description	Models	Modules	Description
H <sub>0</sub>	No modules	Null model; All covariances are hypothesized to be zero	H <sub>4</sub>	Two modules	The first module is bounded by M, MA and M1; The second module is bounded by the rest of the wing
H <sub>1</sub>	Eleven modules	The first module is bounded by P+E encompassing A1 and A2; The second module is bounded by P+E, Cu and M+Cu; The third module is bounded by Cu, M-Cu, MA, M1 and M2; The fourth module is bounded by M, S and MA; The fifth module is bounded by Sc, S, SA and S1; The sixth module is bounded by SA, SP, S1 and S2; The seventh module is bounded by MA and SP; The eighth module is bounded by M+Cu and M2; The ninth module is bounded by M2 and M1; The tenth module is bounded by M2; The eleventh module is bounded by S2 and M1	H <sub>5</sub>	Two modules	The first module is bounded by S, SP and S2; The second module is bounded by the rest of the wing
H <sub>2</sub>	Two modules	The first module is bounded by P+E encompassing A1 and A2; The second module is bounded by the rest of the wing	H <sub>6</sub>	Five Modules	The first module is bounded by P+E encompassing A1 and A2; The second module is bounded by P+E, Cu and M-Cu; The third module Cu, M+Cu, MA, M1 and M2; The fourth module is bounded by M, MA, M1, S2, Sp and S; The fifth module is bounded by Sc, S, SA and S2
H <sub>3</sub>	Two modules	The first module is bounded by Cu and M-Cu; The second module is bounded by the rest of the wing			



**Figure 2.** Models used in the study for the forewings of *I. clypealis*.

The MINT software tests the defined hypotheses if they explain the variation in the data set. These hypotheses generate seven models comprised of modules that represent morphological regions in the wing with the first model as the null hypothesis as shown in Figure 2. The MINT software compares the observed and expected covariance matrices generated from an assumed modular structure of the data [13]. The *Goodness of fit* (GoF) of the models were then tested using the  $\gamma^*$  (Gamma\*) test for GoF statistic which scales linearly the number of inter-module associations in covariance matrices [14]. Low  $\gamma^*$  values and high P-values indicate high degree of similarity between observed and proposed modules [12].

### 3. Results and Discussion

Results shows that model 7 was the best-fit model for almost all of the samples except for the left forewing of male mango leafhoppers from the Paho variety which had the best fit model with model 4 (Table 2). In all tests it was the null hypothesis (model 1) that had the lowest  $\gamma^*$  score and P-value score.

Model 7 hypothesizes that the phenotypic region bounded by the major veins as defined in each module controls the development of the wing. This model compartmentalizes the wing according to its major veins which suggests that wing modularity is regulated by genetic mechanisms that govern the major veins. The Goodness of fit tests suggests that these modules are independent from other modules but are cohesive internally producing the phenotypic structures [15]). In this model, the first module is bounded by the Anal vein encompassing the sub anal veins, A1 and A2. The second module is bounded by Plicus+Emplusal (P+E) vein and the Cubitus (Cu) vein. The third module is bounded by the Cubitus (Cu) and Medial (M) veins. The fourth module is bounded by the Median (M) and Subcostal (Sc) veins. The fifth module is bounded by the Subcostal (Sc) veins.

Model 4 is composed of two modules and is the best-fit model for the left forewing of male mango leafhoppers in the Paho variety ( $\gamma^* = -0.25572, P = 1$ ). This suggests that host plants may affect wing development but these host factors do not seem to affect the right forewing of the male mango leafhopper population whose best fit model is model 7 ( $\gamma^* = -0.31276, P = 1$ ). Model 4 is composed of two modules with the first module bounded by Median and Cubitus veins encompassing the P+E veins and Anal veins. The second module encompasses the rest of the wing.

The consistency of model 7 across host, male and female populations indicates that the modularity of the wing is conserved as the leafhopper infests other hosts. However, the data also imply that this may not be tightly conserved as observed in the left forewing of the male mango leafhopper. Furthermore, the ranking of the other models also vary which may support that wing modularity may be influenced by other factors such as the host.

**Table 2.** Computed  $\gamma^*$ - and P- Values for the forewings of *Idioscopus clypealis*.

Host	Male						Female						
	Left		Right		Left		Right						
Guyabano	Rank	Model	$\gamma$ -Value	P-Value	Model	$\gamma$ -Value	P-Value	Model	$\gamma$ -Value	P-Value	Model	$\gamma$ -Value	P-Value
	1	7	0.3266	0.694	7	0.3151	0.923	7	0.2841	0.912	7	0.2741	0.955
	2	6	0.2997	1	4	0.3103	1	4	0.2436	1	3	0.2378	1
	3	5	-0.244	0.838	6	0.2759	0.997	3	-0.238	1	4	0.2289	1
	4	4	0.2311	1	3	0.2534	1	6	0.2068	0.972	5	0.2119	0.999
	5	2	0.1902	0	5	0.2066	0.91	5	0.2041	0.978	6	-0.209	0.991
	6	3	0.1372	1	2	0.1843	0	2	0.1187	0	2	0.1305	0
	7	1	0	0	1	0	0	1	0	0	1	0	0
Cebu	1	7	0.2987	0.943	7	-0.3009	0.987	7	0.2801	0.998	7	-0.28	0.912
	2	6	0.2566	1	4	0.2696	1	4	0.2716	1	3	0.2502	1
	3	4	0.2383	1	3	0.2604	1	3	0.2352	1	4	0.2202	1
	4	3	0.2298	1	6	0.2599	0.998	6	0.2247	1	5	-0.22	0.992
	5	2	0.1698	0	5	-0.232	0.996	5	0.1874	0.995	6	0.1965	0.977
	6	5	-16909	1	2	0.1311	0	2	0.1435	0	2	-0.163	0
	7	1	0	0	1	0	0	1	0	0	1	0	0
Paho	1	7	-0.2847	0.979	3	-0.2394	1	4	-0.2557	1	7	-0.3128	1
	2	6	0.2539	1	7	0.2386	0.931	3	0.2223	1	4	0.2665	1
	3	4	-0.244	1	6	0.2107	0.995	7	0.2134	0.745	5	0.2582	1
	4	5	0.2132	1	5	0.1663	1	6	-0.172	0.96	3	0.2541	1
	5	3	0.2115	1	4	0.1426	1	5	0.1336	1	6	0.2442	1
	6	2	0.1398	0	2	0.1101	0	2	0.1053	0	2	0.2093	0.002
	7	1	0	0	1	0	0	1	0	0	1	0	0

### 4. Conclusion

The results showed that the forewing of the mango leafhopper is mainly regulated by developmental modules bounded by the major veins. These developmental modules are have conserved across gender, host and left and right wing but may be affected by host.



## Acknowledgement

The senior author would like to acknowledge the Philippine Department of Science and Technology – Accelerated Science and Technology Research and Development Program (DOST-ASTHRDP) for the research scholarship grant.

## Competing Interests

The authors declare that they have no competing interests.

## Authors' Contributions

All the authors contributed significantly in writing this article. The authors read and approved the final manuscript.

## References

- [1] S.F. Gilbert, *Developmental Biology: The Anatomical Tradition*, Sinauer Associates, Inc., Sunderland, MA (2000).
- [2] B. Esteve-Altava, Systematic review of the research on morphological modularity, *bioRxiv*, 027144 (2015), DOI: 10.1101/027144; *Biological Reviews*, DOI: 10.1111/brv.12284.
- [3] M.A. Sturtevant and E. Bier, *Development* **121**(3) (1995), 785 – 801.
- [4] S.F. Gilbert, J.M. Opitz and R.A. Raff, *Developmental Biology* **173**(2) (1996), 357 – 372.
- [5] B. Cui and G. Liu, *International Journal of Biology* **3**(1) (2011), 180.
- [6] Department of Agriculture - Fertilizer and Pesticide Authority (DA-FPA), Registered pesticides for mango as of 01 January 2010, accessed at <http://fpa.da.gov.ph> (2010).
- [7] R.M. Briones, *Market Structure and Distribution of Benefits from Agricultural Exports: The Case of the Philippine Mango Industry*, No. DP 2013-16 (2013).
- [8] Philippine Mango Seedling Farm Corp (PMSFC), Cultural Management, accessed at <http://www.mangoseedling.com/culturalmgmt.html> (2015).
- [9] S.M. Bato, The biology, ecology and control of *Idioscopus clypealis* (Lethierry)(Cicadellidae, Homoptera) [studies conducted in several mango orchards in Southern Tagalog, Central Luzon provinces and at UPLB, Philippines] (1978).
- [10] F.J. Rohlf, *tpsDig, version 2.17*, Department of Ecology and Evolution, State University of New York, Stony Brook (2010).
- [11] F.J. Rohlf, *tpsUtil version 1.44*, New York State University at Stony Brook (2009).
- [12] E.J. Marquez, *Mint: Modularity and integration analysis tool for morphometric data*, version 1, Mammals Division, University of Michigan Museum of Zoology (2008).
- [13] E.J. Márquez, *Evolution* **62**(10) (2008), 2688 – 2708.
- [14] J.T. Richtsmeier, S.R. Lele and T.M. Cole III, Variation: A Central Concept in Biology, 49 – 69 (2005).
- [15] C.P. Klingenberg, *Evolutionary Biology* **35** (2008), 186 – 190.