Developmental modules defining the shape of the forewing of *Scotinophara coarctata*

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ABSTRACT

This study was conducted to determine the possible number of developmental modules defining the shape of the forewing of the rice black bug *Scotinophara coarctata*. The new method of geometric morphometrics (GM) was used and the shapes of the forewings were summarized via Procrustes analysis of a total of 120 landmarks. In this study, Modularity and Integration Analysis was used as a tool to test *a priori* models of variational modularity in multidimensional (mainly morphometric) data in order to analyze the number of developmental modules of the forewings of the insect. Twelve *a priori* models of variational modularity in the GM forewing data were tested using the γ* (Gamma*) test for goodness of fit (GoF) statistic by comparing the observed and expected covariance matrices. Jackknife support values for each model were also computed using γ* as the GoF statistic. The analysis was implemented based on a total of 1000 replicates, dropping 10% of the specimens per jackknife replicate, and computing 95% confidence intervals for the statistic. Results showed that the female RBB forewing is spatially organized into two modules, the partition of clavus and membrane and the corium as an independent structure whereas the male populations exhibit two possible developmental models. These modules are mutually integrated but statistically independent from each other.

**Keywords:** RBB, forewing, modularity, integration, Geometric morphometrics

INTRODUCTION

From its first outbreak in 1982, rice black bug (RBB) *Scotinophara coarcata* until now, had remained as a highly invasive species in many regions in the Philippines. The reports of alarming outbreaks of RBB in many rice-growing sites all over the country and observation that the RBB is a species complex and population differentiation indicate the ability of this pest to conquer various habitats (Barrion *et al.*,1982; Demayo *et al.*, 2007). It is argued that the wings have contributed to the unparalleled success and diversity of this insect thus the study of the RBB flight's evolutionary origin based on its structural design is of general interest. Flight represents a major innovation and the theory that RBB is a species complex based on observed geographical differences can be due to variation in their wing design. This study was therefore conducted to analyze developmental modules of forewings of

RBB. Modularity and Integration (MINT) analysis (Marquez, 2008) was used as a tool to test *a priori* models of variational modularity in multidimensional (mainly morphometric) data in the forewings of the insect specifically the analysis of developmental modules of the forewings. Modules are known also as morphogenetic fields among developmental biologists (Ingham and Martinez, 1992; Williams and Carroll 1993; Gilbert *et al.* 1996; Raff 1996). Fields are distinct from other kinds of modules in that they are morphological units with clear spatial boundaries. The development and morphology of different modules can therefore evolve independently, at least to some extent, without disrupting function at the level of the whole organism.

MATERIAL AND METHODS

Nine-hundred thirteen (913) forewings (left wing: males=249, females=232; right forewing: males=198, females=234) of selected populations of RBB collected from different geographical locations here in the Philippines, as well as a population from Omar, Malaysia. Digital images were acquired from right and left view of each forewing and were obtained with a HP 2400 scanner at 1200 dpi. A total of 120 points were digitized around the outlined of the forewing (Fig.1). X and Y coordinates of the outline data were obtained using tpsRelw [8] before loading to the MINT (Modularity and Integration Analysis Tool) software (Marquez, 2008).
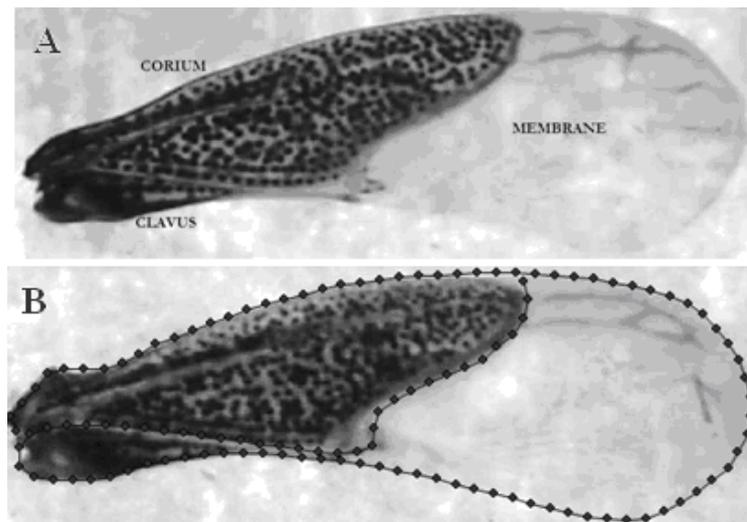
Figs.1. (**A**) Anatomical parts and (**B**) diagrammatic representation of the RBB (*Scotinophara* sp. Stål) forewing indicating the landmarks outlined in this study.

Using the MINT ver. 1.0b software, 4 *a priori* models were constructed with the help of the model building tool option of the software (Table 2). The process generated a total of 12 models of variational modularity in the shape data, including the null model that assumes that no modularity exist (Fig. 2). 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 structure.
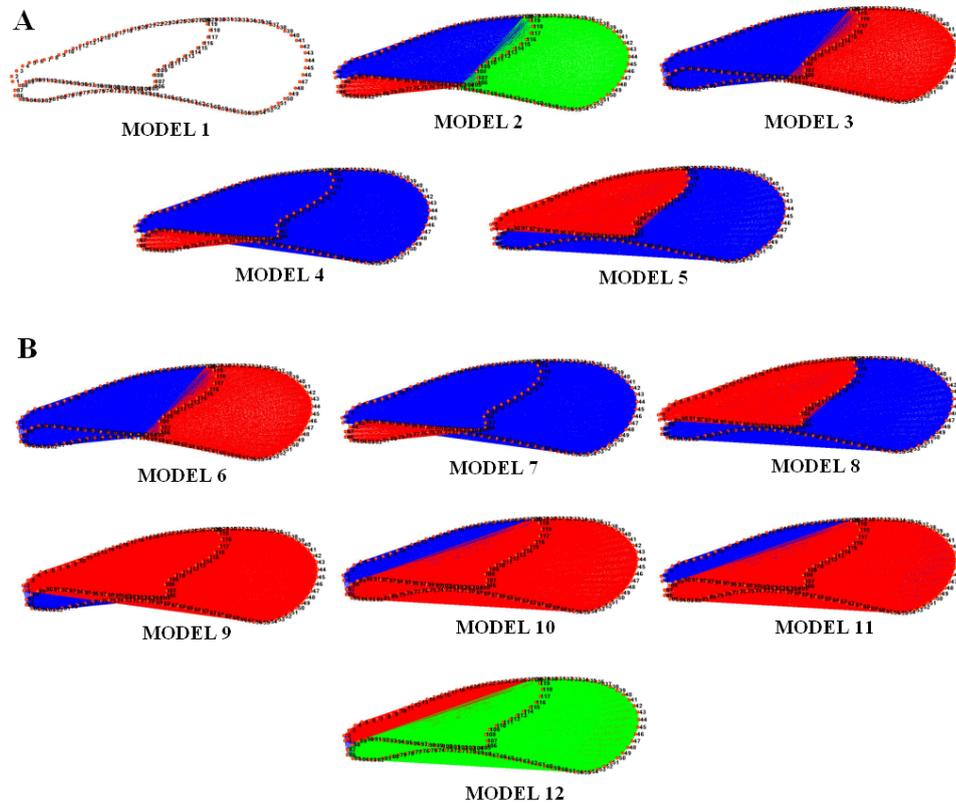
Figs. 2: Models used in this study- (**A**) *A priori*/original proposed models and (**B**) Nonhierarchical/alternative models generated by MINT software.

Table 1: A priori developmental and functional modules of modularity tested in *S. coarctata* forewing.

MODEL		DESCRIPTION
H_0	No Modules	"Null" model, predicting absence of modular structure; all covariances are hypothesized to be zero.
H_1	Corium + Clavus + Membrane	The forewing is divided into three recognizable parts.
H_2	Corium + Clavus; Membrane	The forewing is divided into chitin and membranous material.
H_3	Corium + Membrane; Clavus	Partitions predict integration between corium and membrane.
H_4	Clavus + Membrane; Corium	Partitions predict integration between clavus and membrane.

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 high degree of similarity between the observed data and 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 (Marquez, 2008). The confidence interval for γ^* were obtained using jackknife resampling method (Rohlf, 2006) 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 $\gamma*$) (Marquez, 2008).

RESULTS AND DISCUSSION

Results showed that the best-fit model for the female population which was fairly consistent to all sampled populations was model #5 wherein, the corium and clavus + membrane belong to two separate modules and are differentiated by the presence of chitin and membranous material. Table (2) shows the jackknife support and $\gamma*$ values for male populations computed for each of the 12 models of variational modularity the top 3 best-fit models are listed. While table (3) shows the jackknife support and $\gamma*$ values for female populations computed for each of the 12 models of variational modularity, only the top 3 best-fit models are listed.

The results show that the forewings of the rice black bug (*Scotinophara coarctata*) are organized into developmental modules: {corium and clavus} and {membrane}. These parts are said to be coherent according to their developmental origins, structure and function (Fig. 3). 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 (Klingenberg, 2008).
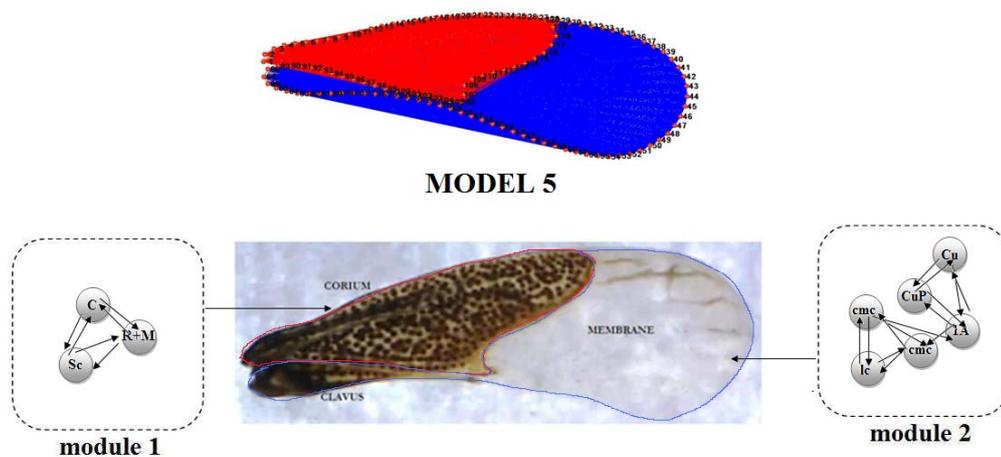

MODEL 5

Fig. 3: Hypothesized possible developmental modules of best fit model#5 of fore- wing of rice black bug (*Scotinophara coarctata*). Component parts within modules are interconnected by many interactions whereas, there are fewer interactions between modules.

The results of this study is also in conformity with the previous thought 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 This is also in line with other studies, suggesting that groups of genes control compartments of wing development. In more general terms, compartments represent individual units of selection and that they are distinct units of selection subjected to different genetic control (Cavicchi *et al.*, 1981; Cowley and Atchley, 1990; Cavicchi *et al.*, 1991; Guerra *et al.*, 1997; Bayle and Penin, 1998; Birdstall *et al.*, 2000;

Zimmerman *et al.*, 2000).

The results of this study also reflect a common genetic basis for the fore wings of the RBB from the Philippines. Further studies are recommended to look into patterns of modularity and integration in some other parts of the insect such as the male and female genitalia. These studies are important especially in assessing the amount of genetic diversity among the populations of RBB from the Philippines.

Table 2: Jackknife support and γ-values computed for each of the 12 models of variational modularity. Only the top three (3) best fit models are tabulated for the male forewing.

POPULATION	n	BEST RANKED MODELS	JACKKNIFE SUPPORT	γ-VALUE	95% CI	P VALUE
LUZON						
Otavi, Bulan Sorsogon	8	5	100.0%	0.19427	0.15447,0.24035	1
		8	100.0%	0.19427	0.15447,0.24035	1
		10	50.1%	0.26021	0.21446,0.30159	0.501
Gadgaran, Matnog, Sorsogon	14	5	100.0%	0.13124	0.10026,0.14267	1
		8	100.0%	0.13124	0.10026,0.14267	1
		10	100.0%	0.18997	0.16124,0.20091	1
Jupi, Gubat, Sorsogon	0	-	-	-	-	-
		-	-	-	-	-
Poblacion, Camarines Sur	17	5	100.0%	0.22353	0.18465,0.24241	1
		8	100.0%	0.22353	0.18465,0.24241	1
		10	85.2%	0.2792	0.24412,0.29293	1
PALAWAN						
Maasin, Brookes Pt. Palawan	16	5	79.8%	0.15354	0.12424,0.17669	0.798
		8	79.8%	0.15354	0.12424,0.17669	0.798
		3	64.9%	0.17122	0.14573,0.20348	0.649
Bonobono, Batarasa Palawan	17	5	100.0%	0.09849	0.075262,0.1299	1
		8	100.0%	0.098491	0.075262,0.1299	1
		10	97.0%	0.15675	0.13362,0.18222	0.97
ILOILO						
Ajuy, Iloilo	40	5	100.0%	0.10955	0.10282,0.11761	1
		8	100.0%	0.10955	0.10282,0.11761	1
		10	100.0%	0.172	0.16559,0.179	1
Dapitan, Pototan, Iloilo	31	5	100.0%	0.12812	0.12087,0.13578	1
		8	100.0%	0.12812	0.12087,0.13578	1
		10	100.0%	0.195	0.18821,0.20121	1
MINDANAO						
Balangao, Zamboang Sibugay	60	5	100.0%	0.08236	0.078905,0.08673	1
		8	100.0%	0.08236	0.078905,0.08673	1
		10	100.0%	0.14281	0.13914,0.147	1
Maranding, Lanao del Norte	69	5	100.0%	0.12571	0.116,0.1391	1
		8	100.0%	0.12571	0.116,0.1391	1
		10	100.0%	0.18684	0.17888,0.19792	1
Agusan del Norte	60	5	100.0%	0.11402	0.10682,0.1306	1
		8	100.0%	0.11402	0.10682,0.1306	1
		10	100.0%	0.17851	0.17047,0.1953	1
Bucac, Bayugan, Agusan del Sur	12	5	100.0%	0.05311	0.050427,0.063106	1
		8	100.0%	0.053105	0.050427,0.063106	1
		10	100.0%	0.10216	0.099149,0.11105	1
Palongalongin, North Cotabato	23	5	100.0%	0.08898	0.083589,0.096268	1
		8	100.0%	0.088976	0.083589,0.096268	1
		10	100.0%	0.14961	0.14277,0.15761	1
New Iloilo, South Cotabato	15	5	100.0%	0.10729	0.10018,0.11988	1
		8	100.0%	0.10729	0.10018,0.11988	1
		10	100.0%	0.17504	0.166,0.18763	1
Gansing, Sultan Kudarat	0	-	-	-	-	-
MALAYSIA						
Omar, Malaysia	22	5	100.0%	0.14934	0.13546,0.16342	1
		8	100.0%	0.14934	0.13546,0.16342	1
		10	100.0%	0.21029	0.19758,0.22243	1

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Table 3: Jackknife support and γ- values computed for each of the 12 models of variational modularity.
Only the top three (3) best fit models are tabulated for the male forewing.

POPULATION	n	Best Ranked Models	Jackknife Support	γ- Value	95% CI	P value
LUZON						
Otavi, Bulan Sorsogon	23	**5**	**100.0%**	**0.17386**	**0.163,0.18336**	**1**
		8	100.0%	0.17386	0.163,0.18336	1
		10	100.0%	0.23618	0.22734,0.24556	1
Gadgaran, Matnog, Sorsogon	33	**5**	**100.0%**	**0.20904**	**0.1901,0.22659**	**1**
		8	100.0%	0.20904	0.1901,0.22659	1
		10	97.9%	0.27163	0.25252,0.28552	1
Jupi, Gubat, Sorsogon	10	**5**	**100.0%**	**0.05013**	**0.030626,0.072906**	**1**
		8	100.0%	0.050129	0.030626,0.072906	1
		3	80.1%	0.092106	0.070922,0.12506	1
Poblacion, Camarines Sur	22	**5**	**100.0%**	**0.12824**	**0.12383,0.13748**	**1**
		8	100.0%	0.12824	0.12383,0.13748	1
		10	100.0%	0.18975	0.18496,0.19763	1
PALAWAN						
Maasin, Brookes Pt. Palawan	11	**5**	**100.0%**	**0.24748**	**0.2172,0.27571**	**1**
		8	100.0%	0.24748	0.2172,0.27571	1
		1	43.4%	0.29588	0.27956,0.33854	0
Bonobono, Batarasa Palawan	14	**5**	**100.0%**	**0.13148**	**0.11054,0.17035**	**1**
		8	100.0%	0.13148	0.11054,0.17035	1
		10	100.0%	0.1947	0.17516,0.2285	1
ILOILO						
Ajuy, Iloilo	30	**5**	**100.0%**	**0.13216**	**0.12128,0.14536**	**1**
		8	100.0%	0.13216	0.12128,0.14536	1
		10	100.0%	0.198	0.18703,0.21227	1
Dapitan, Pototan, Iloilo	22	**5**	**100.0%**	**0.10643**	**0.1008,0.11509**	**1**
		8	100.0%	0.10643	0.1008,0.11509	1
		10	100.0%	0.17211	0.16597,0.1818	1
MINDANAO						
Balangao, Zamboang Sibugay	60	**5**	**100.0%**	**0.14059**	**0.12774,0.15048**	**1**
		8	100.0%	0.14059	0.12774,0.15048	1
		10	100.0%	0.20504	0.19319,0.2137	1
Maranding, Lanao del Norte	69	**5**	**100.0%**	**0.11503**	**0.094357,0.13299**	**1**
		8	100.0%	0.11503	0.094357,0.13299	1
		10	100.0%	0.18208	0.15908,0.19743	1
Agusan del Norte	60	**5**	**100.0%**	**0.13283**	**0.12334,0.1491**	**1**
		8	100.0%	0.13283	0.12334,0.1491	1
		10	100.0%	0.19348	0.18433,0.20749	1
Bucac, Bayugan, Agusan del Sur	17	**5**	**100.0%**	**0.11211**	**0.1017,0.138**	**1**
		8	100.0%	0.11211	0.1017,0.138	1
		10	99.0%	0.18205	0.1697,0.20264	1
Palongalongin, North Cotabato	19	**5**	**100.0%**	**0.15426**	**0.14258,0.16731**	**1**
		8	100.0%	0.15426	0.14258,0.16731	1
		10	100.0%	0.21717	0.20658,0.2275	1
New Iloilo, South Cotabato	18	**5**	**100.0%**	**0.08734**	**0.079918,0.10303**	**1**
		8	100.0%	0.087336	0.079918,0.10303	1
		10	100.0%	0.15096	0.14476,0.16529	1
Gansing, Sultan Kudarat	21	**5**	**100.0%**	**0.12955**	**0.11788,0.15958**	**1**
		8	100.0%	0.12955	0.11788,0.15958	1
		10	85.9%	0.19108	0.17903,0.21885	1
MALAYSIA						
Omar, Malaysia	0	-	-	-	-	-

CONCLUSION

Results showed that the fore wings of the rice black bug is comprised of two mutually exclusive and integrated developmental modules, the {corium and clavus} + {membrane}. These modules are also morphological units with clear spatial boundaries. These modules are said to be tightly integrated resulting from interactions of biological processes. These two are relatively independent from each other and can therefore evolve independently, at least to some extent.

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