Compartmentalization in the shells of four lake populations of *Vivipara angularis*

Conaida G. Camama[1], Mark Anthony J. Torres[2], Muhmin Michael E. Manting[2], Jessie G. Gorospe[3] and Cesar G. Demayo[2]

[1]*MSU- Malabang Community High School, Malabang Lanao del Sur, Philippines*
[2]*Department of Biological Sciences, College of Science and Mathematics, MSU- Iligan Institute of Technology, Andres Bonifacio Avenue, Iligan City,Lanao del Norte, Phillipines*
[3]*School of Graduate Studies, MSU- Naawan, Naawan, Misamis Oriental, Philippines*
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ABSTRACT

This study examinespatterns of modularity or compartmentalization in the shells of Vivipara angularisfrom four populations collected from Lake Dapao at Pualas, Lanao del Sur, Philippines. A total of 120 points were used to outline the margins around the contour of the shell. Modularity and Integration Tool for morphometric data (MINT) software was used to determine compartmentalization or modularity and integration of the shell of V. angularis. Several models were hypothesized and results revealed that the shell of the V. angularis is divided into three distinct modules, namely: the spire, the body whorl and the aperture.It is hypothesized that these distinct modules built from each shell are said to be internally coherent but flexible in their relationships among one another.Since the same pattern of compartmentalization or modularity was observed among the four populations of V. angularis, this was argued to beindicating genetic conservatism in the shell morphology.

Keywords: aperture, spire, modules, modularity, MINT, pleiotropy
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INTRODUCTION

Earlier studies have shown that the shell shape of *Vivipara angularis* varies between sexes [1] and between within-lake populations [2]. The variations observed were in the three distinct and easily recognizable parts of the shell- the spire, the body whorl and the aperture. It is hypothesized that these three parts are separate modules and the development is genetically controlled. In this regard, we examined whether there is pattern of variation within modules as well as associations among them because these modules are hypothesized to be internally integrated by developmental interactions [3-6].Examination of populations of the species will generate information whether or not there is a general pattern of compartmentalization in the shell of the snail. Observed variability in the shell such as shape and size could explain the morphological changes in the organism that tend to have a modular organization [1, 2, 7].

It is argued that organisms are not completely and equally integrated throughout, but they are organized into distinct parts or modules. Modules are traits that are internally coherent but flexible in their relationships among one another

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which are independent from other modules [3].The key property of modular systems is by exhibiting strong interactions within themselves and weak interaction with others [8]. The model of compartmentalization or modularity occurs in developmental, genetic, functional, and evolutionary contexts. Defining modules therefore, can be a best basis to account the variation structure and can identify the homology in the shell's developmental and genetic patterns among populations of *V.angularis*. These types of modularity are mutually influencing each other through various processes within individuals or within populations. Developmental modularity has an effect on both genetic and functional modularity by modulating the available morphological variation while genetic modularity feeds back to developmental modularity through the genetic control of development defines genetic modularity as patterns of joint effects of genes on the traits, which can be represented as a network of pleiotropic relations among traits [9, 10]. Since developmental processes can mediate the expression of genetic variation in phenotypic traits, these two modularity are said to be related. Klingenbergnd Monteiro [11]show that genetic changes can influence developmental modularity by causing alterations in the interactions among the developmental pathways that affect the traits of interest thus it is hypothesized in this study that variation in compartmentalization may exists among the four populations of *V. angularis* in the lake. In this study, we tested several alternative modularity or models of compartmentalization in the shell of *V. angularis* using the Modularity and Integration (MINT) analysis[12].

MATERIALS AND METHODS

V. angularis were collected from the coastal, muddy shore with growing sedges, in the rocks, and in the deepest area of the lake from the four locations around the vicinity of Lake Dapao, Philippines (Fig. 1). A total of 240 samples, 60 samples in each four locations were photographed with a mounted digital camera on its aperture face (ventral)oriented in such a way as to be able to show both the outer and inner edges of the aperture lip. Outlines of curves were digitized using TpsDig2[13]. A total of 120 points were digitized around the generated outline of the ventral portion of the shell (Fig.2). All points were digitized in the same order and always resample to maintain consistency within the populations. The data were then loaded to the MINT (Modularity and Integration Analysis Tool) software version 1.5 [14] to test the acceptability of the hypothetical modules which assumes that the data have a modular structure. Based on the modified data resulting from partitioning, the entire data space into orthogonal subspaces of modules, the covariance matrices were then computed which are expected under models of modularity. A total of 4 *a priori* model was constructed (4 models 2-5) (Fig.3). Table 1 shows the criteria for the choice of landmarks to be included in modules within every model of *V. angularis*. The alternative models of modularity were tested against the landmark datasets, with the purpose of finding the module that accounts for most of the covariation of structures in the datasets.
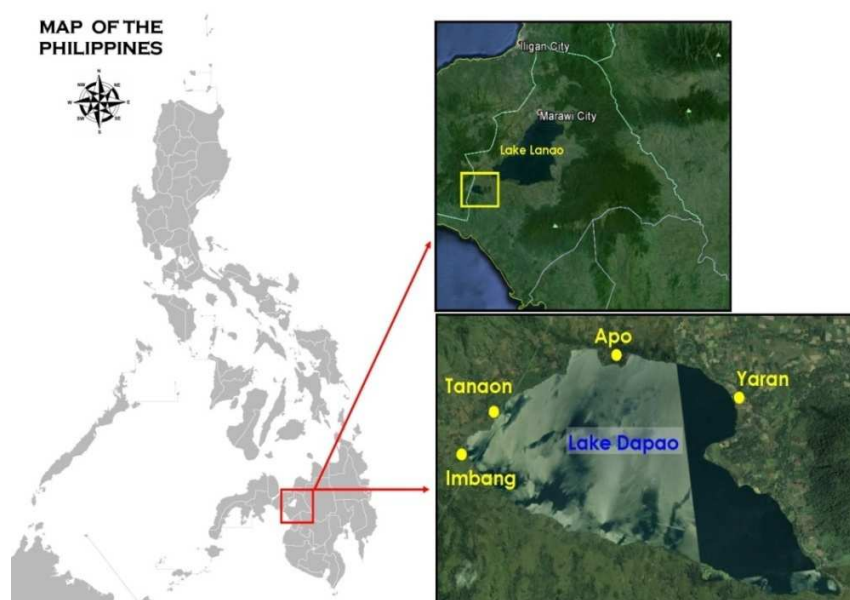

Figure 1. Map showing the study area Lake Dapao in Pualas, Lanao del Sur, Mindanao, Philippines
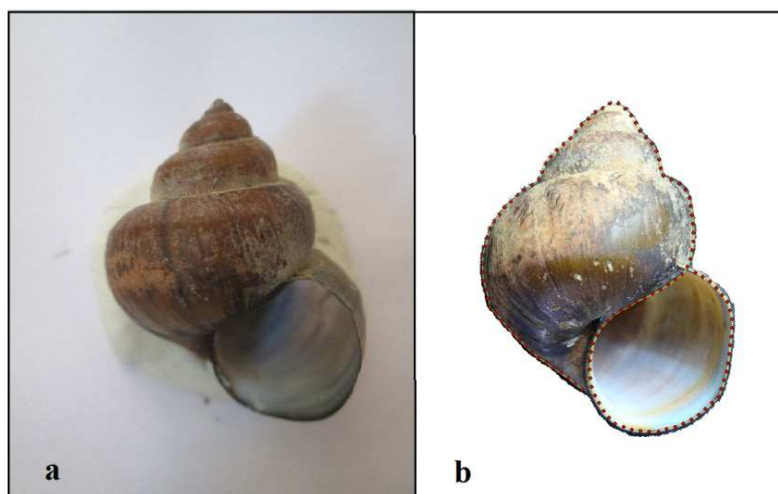*Source: http://maps.google.com/maps?hl=en.*

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Figure 2. a) Digital image of *V. angularis*, b) Outline data of *V. angularis* at 120 points

Table 1. *A priori* models tested in the study of *V. angularis*.

	Models	Description
H_1	No module	"Null" model, predicting the absence of modular structure; all covariances are hypothesized to be zero.
H_2	1 module	Only one module was considered in this model. The spire, body whorl and apertural region as one.
H_3	2 modules	The spire and body whorl are considered as one module; the apertural region as the second module.
H_4	2 modules	The spire represents one module; the body whorl and apertural region as the second module.
H_5	3 modules	The spire represents one module; the body whorl as the second module; and the apertural region as another module.

Goodness of fit (GoF) between expected and observed covariace matrices was assessed by using the gamma value (γ^*).The interest of using the MINT software, is to test the GoF models depicting tight associations within integrated sets of traits, the variational modules and no associations with traits outside of those sets. P-values and γ-values were assumed to be the results of this GoF tests. The computed γ^*values were subjected to a Monte Carlo randomization test in which model covariance matrix and the original sample size of each model were used to parametize a Wishart distribution[12]. The γ^*values were used to determine if the best–fitted model is the one from where the data was derived when fitted to the original set of models and to complete set of possible model combinations of the *V. angularis* shell. A low P-value (P<0.05), closer to 0, indicates that the models generated are no longer different from the null model and thus a poorly fitting model. However, models which corresponds to large values of γ^*, indicating a large difference between observed data and proposed model thus corresponds to the best fitted model [12, 15].

Jackknife support was used in determining the best-fitting models. Jackknife support values were determined by re-sampling a total of 1000 replicates using γ^* as the GoF statistic, randomly dropping 10% of the individuals in each replicate and computing 95% confidence intervals for the GoF statistic. Jackknife support, which is a measure of model support and whose values equal to 1 or closer to 1, indicates that each of the 1000 jackknife replicates for which a γ^* value was calculated, the model ranked as number 1 is the best fitting alternative [12, 16].

RESULTS AND DISCUSSION

Figure 3 shows five alternative models (6-10) that resulted after individual defined models within loaded modules (models 1-4) were mixed by MINT (Fig. 3). Comparing *a priori* and the alternative models generated, model 5 and 6 were the same, so with 2 and 11, 3 and 9, 4 and 10 (Fig. 3). Monte Carlo test yielded P- and gamma (γ^*) values (Table 2) which depicted associations within integrated sets of traits or variation modules show the top three models in the compartmentalization of the shells of the four *V. angularis* populations (Tables 2 and 3). The best-fit model among the *a priori* models of four populations of *V.angularis* is model 5 (same as the alternative model #6) which hypothesizes that the spire, the body whorl, and the apertural region were distinct modules controlled be genes sets where each module affect developmental and genetic modularity in the *V. angularis* shell. This means that each module is a unit that is tightly integrated internally but relatively independent from other modules [9]. This study is in conformity to a number of studies suggesting that the shell of *V. angularis* is divided into compartments each of

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which is a separate developmental module. Compartments represent individual units of selection and that they are distinct units of selection subjected to different genetic control [15, 17-20]. This study also show that there is a fair consistency in the best-fit models for all the populations and in the pattern of developmental modules which may imply that the shell of *V. angularis* is highly conserved following the same pattern of development. Consistency also suggests that there is not much variation in the arrangement of developmental modules between populations of *V. angularis*.
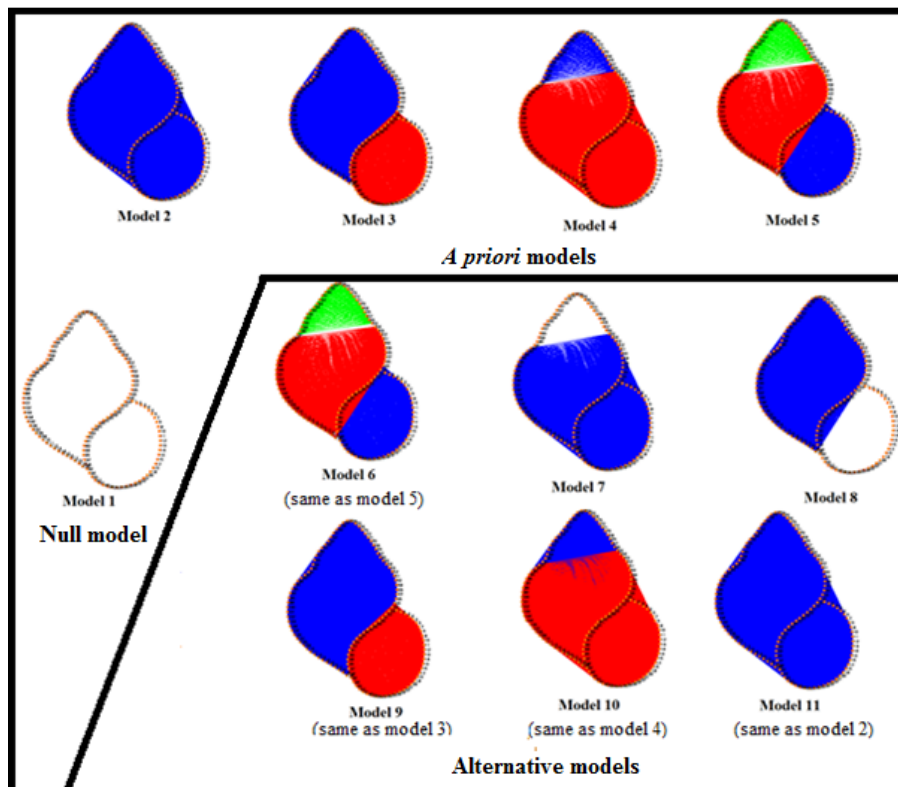

Figure 3. Model 1 (hypothetical model); 2-5 (*a priori* models) and models 6-11 (alternative models)

The existence of another best-fit model #7 (an alternative model) which hypothesizes only one possible module for the spire and body whorl but no module for the aperture (Figure 4), may indicate evidence of developmental interactions [21], or maybe phenotypic plasticity or factors that act during ontogenetic development [22-23]. The differences in the number of modules shown by the best-fit 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 [24] or, there were lack of physical communication between them and/or from the shell-cell-specific genetic composition [25].

Table 2. Modularity and Integration results of Pooled data of four populations of *V. angularis*

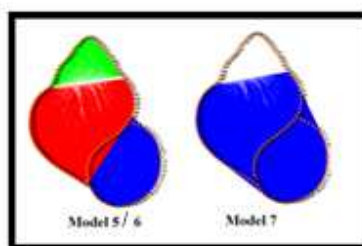| Model	Rank	γ – value	P- value
5	**1**	**0.091318**	**0.997**
6	**1**	**0.091318**	**0.997**
7	2	0.11680	1

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Figure 4. Top best fit models for the four populations of *V. angularis*

Table 3. Modularity and Integration results of the shell of each*V. angularis* populations

Population	Model	Rank	γ- value	P- value
Apo	**5**	**1**	**0.10304**	**0.995**
	6	**1**	**0.10304**	**0.995**
	7	3	0.11375	1
Imbang	**5**	**1**	**0.12286**	**0.964**
	6	**1**	**0.12286**	**0.964**
	7	3	0.1398	1
Tanaon	**5**	**1**	**-0.04962**	**1**
	6	**1**	**-0.04962**	**1**
	7	3	0.007678	1
Yaran	**5**	**1**	**0.095046**	**0.996**
	6	**1**	**0.095046**	**0.996**
	7	3	0.12893	1

CONCLUSION

Results of this study show the shell of *V.angularis* is divided into three distinct compartments namely: the spire, the body whorl and the apertural regionrepresenting individual developmental modules controlled by different gene nets. While there was the existence of an alternative best-fit model, this indicatesinteractions and influences among the developmental modulesaffecting functional and genetic interactions transformedinto phenotypic variations.

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