

# Modularity and integration analysis of rice seed using MINT ver. 1.0b software

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**Abstract.** The study of phenotypic integration concerns the modular nature of organismal phenotypes. The concept provides for a rationale on the observation that certain subsets of phenotypic traits show particularly high levels of association over development and/or evolution. The rice seed consists of morphological parts that can be argued to be a good model for modularity and integration analysis thus this study was conducted. Patterns of phenotypic integration and modularity in the mature seeds of weedy rice using the Modularity and Integration Analysis Tool (MINT) ver. 1.0b were used in this investigation. A total of 8 variational modularity models in the shape data were tested using the  $\gamma^*$  (Gamma\*) test for goodness of fit (GoF) statistics. This was used to compare the observed and expected covariance matrices and implemented based on a total of 1,000 replicates. Jackknife support values for each model were also computed using  $\gamma^*$  as the GoF statistic. Results revealed that the best fitted model showing the mature rice seed is organized into two statistically independent modules, one controlling for the lemma and the other for the palea. This model has a standard gamma value of 0.18575 and a P value of 1 based on a Monte Carlo test with 1000 replicates. The observed partitioning of the co-variation of morphological data into statistically independent blocks or units suggests the presence of modules in the rice seed.

**Key Words:** modularity, integration, jackknife, lemma, palea.

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## Introduction

Phenotypic variations are not only observed in the gross morphology of an evolving rice plant but also present in smaller organs like the rice seed. Different species and varieties of rice including different populations of weedy rice display continuous variations of seed phenotype as a result of complex interplay of gene flow, recombination and selection. Along the length of chromosome, blocks of genes (construed as QTL) with direct involvement in shaping up phenotype of a quantitative trait often have extensive linkage and/or pleiotropy which result to high genetic correlations that is manifested phenotypically through co-variations of traits. One aspect that remains unclear in the case of rice seed is whether this co-variation is generalized affecting the whole seed or there is a pattern of localized strong co-variation affecting only specific functional structure or group of structures that are called as modules (Magwene 2006). Modules are defined by the strength of integration among traits, and the magnitude is expressed mathematically in terms of correlations and co-variations of traits. Highly interacting traits have high co-variation and correlation values indicating strong integration that often result to formation of modules. Traits that belong to different modules are not interacting so well, and thus have weak integration and less cohesive than traits within a module (Magwene 2006). The presence of modules and their

recognition as an evolutionary unit is established such as the skull of peramelid marsupial, *Echymipera kalubu* (Goswami 2007) and the wings of Bumble bee (Klingenberg & Leamy 2001). Similarly, the rice seed has independent structural and functional parts whose spatial pattern is practically similar to the conceived modules.

Basic structural parts of the rice seed that may be considered as modules are the lemma, palea, sterile lemma, and sterile palea. These two later sterile structures attached to the base of the seeds at the rachilla are reduced in size through its evolutionary history because they are non functional. The less obvious part which are slightly raised and sometimes colored at the tip of both lemma and palea are the apiculi, assumed here in the current study as part of lemma and palea. Similarly, the pointed structure attached to the lemma apiculus is the awn. This structure is present in some species but absent in others, and thus eliminated in the study.

The concept of modularity and integration have been tested in various body parts of organisms such as the mouse mandible (Klingenberg 2008), skull of peramelid marsupial, *Echymipera kalubu* (Goswami 2007), wings of bumble bee (Klingenberg & Leamy 2001), *Drosophila melanogaster* (Klingenberg & Zaklan 2000), tsetse flies *Glossina palpalis* (Klingenberg & McIntyre 1998) and others.

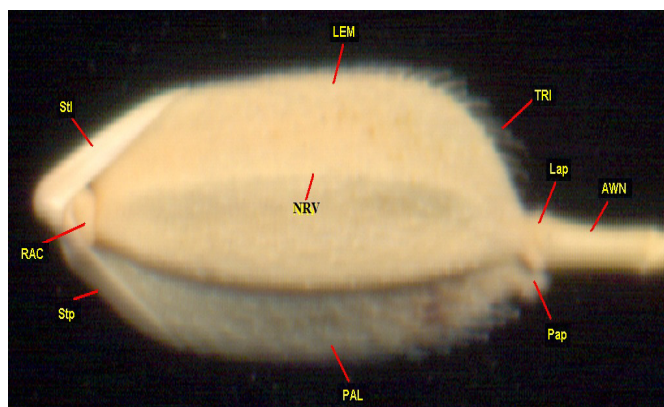


Figure 1. Rice seed and its parts. Lap-lemma apiculus, LEM-lemma, Stl-sterile lemma, RAC-rachilla, Stp-sterile palea, PAL-palea, Pap-palea apiculus and TRI-trichomes.

The two concepts were also tested at the molecular level, and were found that molecular networks also follow patterns of integration and modularity (Tanay *et al* 2004).

In this study, we used the Cartesian (x and y) coordinates as the morphological data to explore patterns of trait integration and to determine the presence and number of modules in the rice seed. These coordinate data from the outline of the rice seed were considered here as characters or trait variables in which their co-variations are measured. On the assumption that there is partitioning of data space in the co-variation matrix, the orthogonal subspaces based on the strength of co-variation create a structuring that defines modules in the rice seed. These data structure was compared to the hypothesized models and statistically tested whether it could support the different hypothesized alternative models.

## Material and Method

### Sample Preparation and Data Collection

One population of weedy rice from Philippine Rice Research Institute (PhilRice) consisting of 94 samples of seeds were air dried and scanned at 2400 dpi. Samples were then digitized by locating a series of 100 points along the margin of the curve using tpsDig software developed by Rohlf (2005).

Landmark data of the 100 points along the seed outline were extracted using tpsUtilities developed by Rohlf (2005). These landmark data were the cartesian (x and y) coordinates of the points. These were then loaded into Modularity and Integration Analysis Tool (MINT) ver. 1.0b for the generation of variational modularity models (Marquez 2008b).

### Model Construction and Model Testing

Landmark coordinates as data of the seed outline were loaded into the Modularity and Integration Tool (MINT) software ver 1.0b (Marquez 2008b). Using the model building tool option of the MINT software, 4 a priori models were constructed including the null model which assumes absence of co-variations among traits. Modules in every model were constructed based on the natural existing structural and functional parts of the rice seed, which were products of genetic interactions and developmental processes.

MINT combined the 4 a priori models and generated 4 non-hierarchical alternative models. Every model generated was considered as hypothesis in the study. Model 1 represented the null model which assumed absence of modules, and models 2, 3 and 4 were a priori models (Figure 1A). Models 5, 6, 7 and 8 were MINT generated non-hierarchical alternative models (Figure 1B). Descriptions of these hypothesized models are given in Table 1.

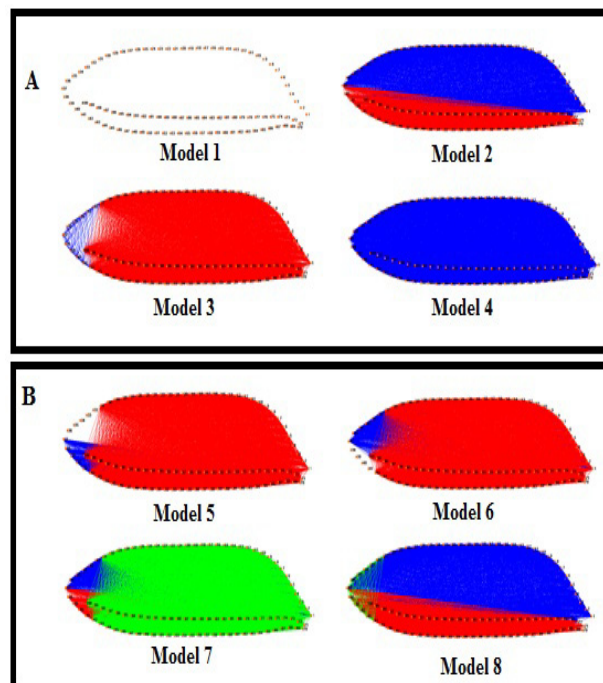


Figure 2. Hypothesized models. A) model 1 represents the null model, and models 2, 3 and 4 are a priori models. B) models 5 to 8 are MINT generated non-hierarchical alternative models.

The best-fitted models were determined using jackknife support. The jackknife support values for each model were computed by resampling 1000 replicates using  $\gamma^*$  as the GoF statistic, dropping 10% of the specimens per jackknife replicate, and computing 95% confidence intervals for the statistic. Jackknife support, which is a measure of model support, was computed by counting the proportion of Jackknife samples in which a model ranks first (Marquez 2008a).

## Results and Discussion

Results of the modularity and integration analysis are shown in Table 2. Three models of the seed modules were found to have P values of 1.0. These three models in the following ranks  $2 > 8 > 3$  were supported highly by the data as indicated by relatively low Gamma ( $\gamma^*$ ) values and high P values. Very low Gamma value approaching zero means that the difference between the observed data and the model is very small and negligible, and usually have large P value. This statistical relationship indicates that both the model and the observed data are statistically the same. Although models 2, 8 and 3 were supported by the data only model 2 is considered the best fitted model (Fig. 3). Model 2 came out as the best fitted model to the data after the test of Goodness of Fit (GoF) and Jackknife resampling at 1000 iterations. Model 2 obtained the lowest  $\gamma^*$  (Gamma) value and highest Jackknife support values of 100% (Table 2).

Table 1. Descriptions of the functional, genetic and developmental modules of the hypothesized a priori models for the rice seed

	Model	Landmark points	Descriptions
<b>HO</b>	Model 1 as the null model	No modules	This “null model” assumes absence of modules and that interactions or co-variations among traits (landmarks) are hypothesized to be zero.
<b>H1</b>	Hypothesized model 2 consisting of 2 modules	(1-37) (72-100)	This model divided the rice seed into 2 functional compartments or modules: the upper large compartment called lemma including its sterile lemma; the second compartment which is smaller than lemma is the palea that includes sterile palea at the posterior end of the seed.
<b>H2</b>	Hypothesized model 3 consisting of 2 modules	(1-31, 42-100) (31-42)	Divides the seed into 2 functional compartments or modules: 1 <sup>st</sup> module is the seed covering compose of lemma and palea. 2 <sup>nd</sup> module is composed of the reduced structures at the posterior end of the seed called sterile lemma and sterile palea.
<b>H3</b>	Hypothesized model 4 consisting of a single module	(1-100)	This is a single module model in which all structural and functional compartments which are the lemma, palea, sterile lemma and sterile palea are included

Table 2. Modularity and integration result for the rice seed

Model	Rank	Γ* value	95% CI		P-values	% JSV
			LV	UL		
model 2	1	0.186	0.180	0.194	1	100
model 8	2	0.244	0.234	0.254	1	92
model 3	3	0.249	0.249	0.249	1	88
model 4	4	0.259	0.251	0.271	0.998	86
model 5	5	0.269	0.260	0.279	0.997	92
model 6	6	0.284	0.276	0.292	1	66
model 7	7	0.288	0.272	0.301	0	46
model 1	8	0.294	0.288	0.302	0	76

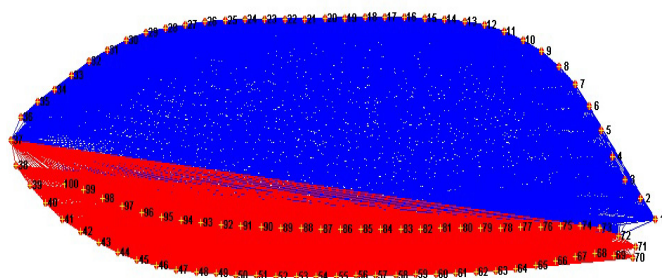


Figure 3. Best fitted model (model 2) showing two functional structural compartments as a product of genetic and developmental processes.

This model describes the mature rice seed is composed of two modules, module 1 is the lemma including its sterile lemma at the posterior end of the seed, and module 2 which is the palea including the sterile palea at its posterior end. While model 8 and model 3 after 1000 times randomization using Monte Carlo test also has P=1, Jackknife support values to the model based

on probability from jackknife samples are however lower than model 2. This result was expected since weak co-variation between modules also occur, thus more than one modular pattern may be supported by the data.

The covariance values generated in this analysis and used as a measure of interactions among variables reflect interactions of different developmental processes (Breuker *et al* 2006). Those that interact highly normally have tight associations. As shown in Figure 2, landmarks 1 to 37 and 72 to 100 exhibit high levels of interaction implying that variables are highly associated with each other making up a single independent module (corresponding to the lemma) (Magwene 2006; Klingenberg *et al* 2004) and landmarks 37 to 100 constitutes another module (corresponding to palea). It is important to note that modules can act as genetic constraints (Klingenberg 2008). In the case of rice seed, modularization and constraints may have its origin at the level of spatiotemporally dynamic interactions between genes, cells during development (Ciudad 2007). Modular constraints could be the reason why despite the large variation in rice seeds yet overall rice seed morphology is the same (Jaranilla *et al* 2008).

It appears then that presence of modules help in the canalization of traits in the rice seeds. Pleiotropy, which refers to the action of different alleles of the same gene, may also produce variations within the module and is an attractive explanation for the variations observed in the rice seed (Klingenberg 2008; Gillespie 1984; Barton 1990; Zhang *et al* 2002). Modularization of morphological structures in the rice seed can be attributed to the reduction of pleiotropy between the lemma module and the palea module thus the effect is largely confined within module (Hansen 2003).

## Conclusions

The observed partitioning of the co-variation of morphological data into statistically independent blocks or units suggests the presence of modules in the rice seed. Modularization of morphological structures in the rice seed can be attributed to the reduction of pleiotropy between the lemma module and the palea module thus the effect is largely confined within module.

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