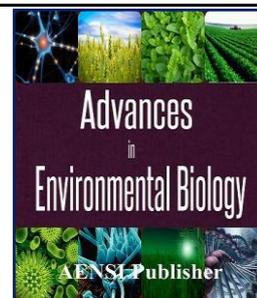




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## Determination of Developmental Modules of the Fore and Hind wing of the Peacock Pansy *Junonia almanac* Linnaeus 1758 (Nymphalidae: Lepidoptera) using Modularity and Integration software

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### ABSTRACT

Parts of an organism are coordinated to form a functional whole. These are referred to as modules. Modules are units within a developmental system that are defined by their internal coherence and relative independence from other parts of the system. Butterfly wings are composed of compartments divided into several veins. These compartments have sets of genes that are correlated to serve the function of a wing either it's the fore or the hind wing. In this study, developmental modules in the fore and hind wing of the Peacock Pansy butterfly (*Junonia almanac*) was determined by analyzing seven priori models using the MINT software. A total of 195 points were used to trace and outline the margins of the wings as well as the major veins. The test was conducted based on a Wishart/Monte Carlo test on a total of 1,000 replicates. Different pattern of developmental modules were found indicating the presence of sexual dimorphism and variations caused by factors of modularity. Results also suggest that the compartments of the wings are autonomous unit of morphological variation and that they correspond to distinct cell lineages and domains of gene expression.

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## INTRODUCTION

Lepidopteran wings exhibit great diversity in design and in colour, with the pattern of almost every species being distinct from all others. Patterns on the dorsal and ventral wing surfaces are frequently quite dissimilar and those of the forewing and hindwing are also different. In addition some species are genetically colour-polymorphic while others show seasonal polyphenism or phenotypic plasticity, by which individuals with similar genotype can develop different patterns in response to rearing conditions [1]. The development of the wing pattern elements is to some extent integrated in overall wing development meaning the butterfly wings experience a multitude of different natural and sexual selection pressures either simultaneously or sequentially, which will select for a particular wing size and shape in response to the environmental and ecological conditions experienced [2]. The elements have identities that can be traced from species to species, and typically across genera and families. Because of this, it is possible to recognize homologies among pattern elements and to study their evolution and [3]. Because it displays diversification, it is an ideal material to study morphological integration and the evolution of developmental independence [4].

In this study, the morphometric data of the fore- and hind wing of the Peacock Pansy butterfly (*J. almanac*) was examined to determine how many modules are found between the sexes and are they morphologically integrated. This was tested using Modularity and Integration (MINT) analysis tool [3]. The patterns of variational modularity and integration are assessed by testing alternative *a priori* models, each of which hypothesizes a distinct modular structure caused by specific functional or developmental mechanisms [3].

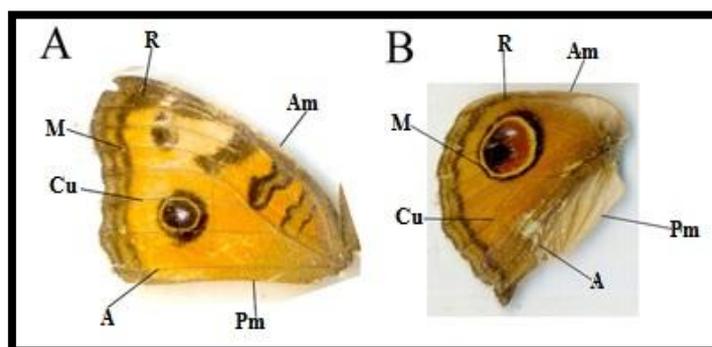
## MATERIALS AND METHODS

Samples were collected from 2 provinces in Mindanao: Lanao del Norte, and Misamis Occidental, using a lightweight, long handled sweep net. The collected butterflies were then placed delicately in a white paper

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envelope to prevent damages. Identification of the individual were based on the following: In males, they are smaller which means less weight to carry, greater strength to weight ratio for better agility, they are more brightly colored, more pointed forewings (flight aerodynamics, built for speed, chase and evasion) and they have skinner abdomens (no eggs) which is opposite to the females.

The fore and hind wing were carefully detached from the thorax using a dissecting needle or scalpel and mounted on two glass slides using forceps and sealed together using invisible tape and then labeled. The mounted wings were scanned using a Hewlett-Packard Jacket 2400 scanner in a 1200bpi resolution. The images was then cropped, sorted according to wing type and orientation and then saved. A total of 195 points was used for outlining the shape of wings as well as their major vein pattern. TPSDig2 software [5] was used in digitally outlining the fore and hind wing. The outlined data was then converted to landmark points (XY) using TPS util[6] and then loaded to MINT (Modularity and Integration Analysis Tool). Different hypothesis (Tables 1-2a, 2b) were formulated to test and determine whether the entire wing of *J. almanais* a single module or whether the compartments are independent units.



**Fig. 1:** Wing venation pattern of butterfly of *J. almana* Linnaeus A) fore- and B) hind wings: Am= Anterior margin, R= radius, branched, M= Media, Cu= Cubitus, A= Anal vein, Pm= Posterior margin

Table 1. Developmental modules of the fore wings based on the wing venation pattern of *J. almana*L.

Model	Modules	Descriptions
H1	no modules	Null model, there is no compartmentalization within the wings
H2	1	Every compartment serves as one
H3	5	between the anterior margin of the wing and the 2nd radial vein between the 2nd radial vein and the 4th radial vein between the 4th radial vein and the 2nd medial vein between the 2nd medial vein and the 2nd cubitus vein between the 2nd cubitus vein and the posterior margin of the wing
H4	5	between the anterior margin of the wing and the 1st radial vein between the 1st radial vein and the 2nd radial vein between the 2nd radial vein and the 2nd cubitus vein between the 2nd cubitus vein and the anal vein between the anal vein and the posterior margin of the wing
H5	3	between the anterior margin of the wing and the 2nd radial vein between the 2nd radial vein and the 2nd cubitus vein between the 2nd cubitus vein and the posterior margin of the wing
H6	4	between the anterior margin of the wing and the 1st radial vein between the 1st radial vein and the 4th radial vein between the 4th radial vein and the 1st cubitus vein between the 1st cubitus vein and the posterior margin of the wing
H7	2	between the anterior margin of the wing and the 1st medial vein between the 1st medial vein and the posterior margin of the wing

**Table 2a:** Developmental modules of the hind wings based on the wing venation pattern of *J. almana*L.

Models	Modules	Descriptions
H1	no modules	Null model, there is no compartmentalization within the wings
H2	1	Every compartment serves as one
H3	5	between the anterior margin of the wing and the 2nd radial vein

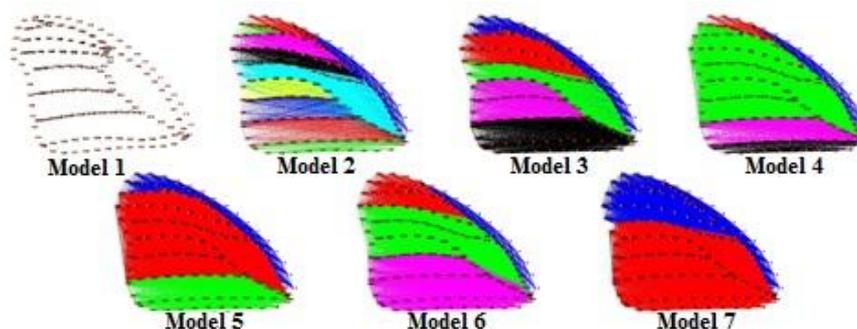
**Table 2b:** Developmental modules of the hind wings based on the wing venation pattern of *J. almana*L.

Models	Modules	Descriptions
H3		between the 2nd radial vein and the 1st medial vein between the 1st medial vein and the 1st cubitus vein between the 1st cubitus vein and the anal vein
H4	5	between the anal vein and the posterior margin of the wing between the anterior margin of the wing and the 1st radial vein

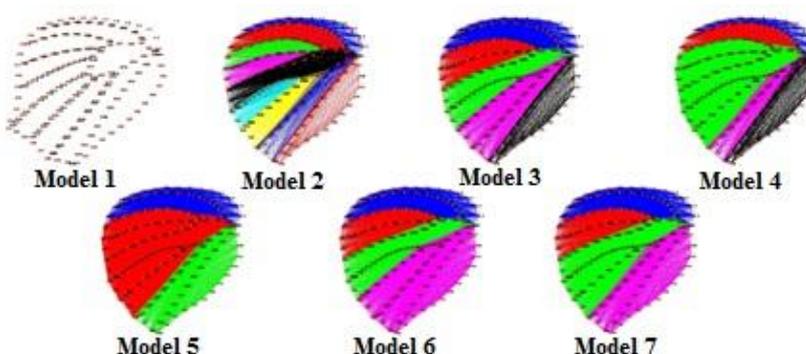
		between the 1st radial vein and the 2nd radial vein
		between the 2nd radial vein and the 2nd cubitus vein
		between the 2nd cubitus vein and the anal vein
		between the anal vein and the posterior margin of the wing
H5	3	between the anterior margin of the wing and the 2nd radial vein
		between the 2nd radial vein and 1st cubitus vein
		between the 2nd cubitus vein and the posterior margin of the wing
H6	4	between the anterior margin of the wing and the 2nd radial vein
		between the 2nd radial vein and the 1st medial vein
		between the 1st medial vein and the 1st cubitus vein
		between the 1st cubitus vein and the posterior margin of the wing
H7	4	between the anterior margin of the wing and the 2nd radial vein
		between the 2nd radial vein and the 1st medial vein
		between the 1st medial vein and the 2nd cubitus vein
		between the 2nd cubitus vein and the posterior margin of the wing

The models were tested using the Modularity and Integration Tool version 1.5 [3] to test for variation modularity to evaluate whether a proposed model or hypothesis is good enough to explain variation in the data set. The models are outlined using tpsDig tool. The process generated a total of 14 models of variational modularity in the shape data, including the null model that assumes that no modularity exist (Fig. 2-3). The points representing the modules are converted to landmark. Each model represents a hypothesis.

Resulting P-values and  $\gamma^*$  values depict associations within integrated sets of traits or variational module. A low (<0.05) P value, closer to zero, indicates that the models generated are significantly different from the observed data. The model is thus a poor fit and must be rejected. However, P-values greater than 0.05, ( $P > 0.05$ ) and approaching 1, correspond to low  $\gamma^*$  values. This indicates a high degree of similarity between the proposed model and the observed data and thus, the proposed model is accepted [3].



**Fig. 2:** Models used in this study for the fore wings of Peacock Pansy, *J. almanaL*.



**Fig. 3:** Models used in this study for the hind wings of Peacock Pansy, *J. almanaL*.

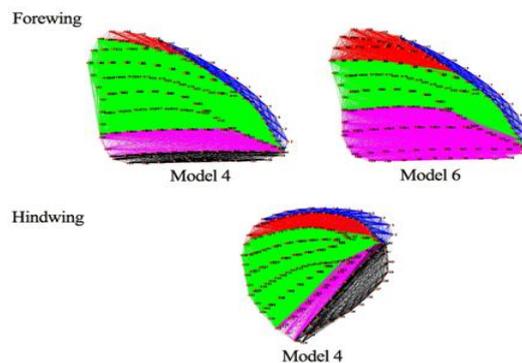
## RESULTS AND DISCUSSION

The best fit models of the left fore wing of *J. almana* are model 6 and 4 for female and male, respectively while in the right fore wing is model 4. The best fit models of the left and hind wing of *J. almana* is model 4 for the female and male. The two competing models are both accepted with P-values greater than 0.05 and being the best fit models, both have the lowest  $\gamma^*$ -value. Figure 4, Model 4 showed the best fit of majority. Model 4 of the fore and hind wing has 5 distinct modules (1) the anterior margin of the wing is bounded by the 1st radial vein

(2) the 1st radial vein is bounded by the 2nd radial vein (3) the 2nd radial vein is bounded by the 2nd cubitus vein (4) the 2nd cubitus vein is bounded by the anal vein (5) the anal vein is bounded by the posterior margin of the wing. The result is supported by the Gamma values presented in Table 8. The P-value for the null hypothesis that the data are no more different from this model than expected by chance is 1 based on Wishart/Monte Carlo test with 1000 replicates.

**Table 3:** Best Fit Model for fore and hind wing of *J. almana*L.

	Sex	
	Female	Male
Forewing		
Left	6	4
Right	4	4
Hindwing		
Left	4	4
Right	4	4



**Fig. 4:** Best model of fore- and hind wings of *J. almana*L.

**Table 4:** Top three best fit models for the left and right Fore- and Hind wings of male and female *J. almana*L.

Forewing									
Female					Male				
Wing	Model	Rank	Gamma Value	P - Value	Wing	Model	Rank	Gamma Value	P - Value
Left	6	1	0.14474	1	Left	4	1	0.12489	1
	4	2	0.15322	1		6	2	0.13051	1
	5	3	0.1591	1		5	3	0.14091	1
Right	4	1	0.15565	1	Right	4	1	0.20129	1
	5	2	0.18479	1		5	2	0.23775	1
	6	3	0.21456	1		6	3	0.2729	0.999
Hindwing									
Female					Male				
Wing	Model	Rank	Gamma Value	P - Value	Wing	Model	Rank	Gamma Value	P - Value
Left	4	1	0.22931	1	Left	4	1	0.2378	1
	5	2	0.24772	1		5	2	0.25848	1
	7	3	0.30427	1		3	3	0.34111	1
Right	4	1	0.1832	1	Right	4	1	0.39882	1
	5	2	0.2055	1		5	2	0.41106	1
	3	3	0.29725	1		3	3	0.42942	0.849

The results shows (Table 4) that the left fore wing have different ranks of best fit model while on the right showed the same ranks. The same is seen on the hind wings. The left hind wing showed different ranks of best fit models while on the right showed the same ranks. This difference in the best fit model indicates factors caused by modularity and sexual dimorphism. There is covariation because the compartmentalization process creates modules that are overlap spatially, even if the process responsible for it is independent. Sexual dimorphism evolved due to independent effects of genetic variation on both sexes. Sex specific gene regulations occur as a result of selection during male and female towards different reproductive optima. Changes in gene regulations and expressions may have further consequences for the sexual fitness of one or both sexes [7].

The results also confirmed a number of studies suggesting that insect wings are divided into compartments and that their modules served as autonomous unit of morphological variation and each of compartments are a separate developmental module [4, 8, 9, 10, 11] These compartments may correspond to distinct cell lineages and domains of gene expression[12, 13]. This aspect of variational modularity describes the condition of varying

in modular fashion and results from the operation of several unique evolutionary processes that operate on phenotypic development [14].

#### Conclusion and recommendation:

This study was conducted to determine the modularity and integration in the fore- and hind wings between sexes of the Peacock Pansy Butterfly, *J. almana*. and is limited only on the samples collected from 2 provinces in Mindanao: Lanao del Norte and Misamis Occidental. A total of 14 models of variational modularity in the shape data; including the null model that assumes that no modularity exist, was conducted and tested to determine how many modules are found between the sexes of the fore and hind wing of *J. almana*.

Results show various ranks of best fit models indicating presence of sexual dimorphism and variations caused by factors of modularity. Sexual dimorphism caused by sex specific gene regulations and variational modularity associated with the genetic material variation and environmental condition influence.

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