

Describing Developmental Modules of Modularity and Integration in the Fore and Hind Wings of the Common Evening Brown Butterfly *Melanitis leda* Linnaeus (Nymphalidae: Lepidoptera) Using MINT Software

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ABSTRACT

In this study, developmental modules of modularity and integration of the fore and hind wings of *Melanitis leda* were described using the MINT software (Modularity and Integration Tool, ver. 1.5, Marquez, 2008). 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. The best fit models are determined with the highest P-value and lowest gamma value. Differences in ranks of the top 3 models were observed in both the fore and hind wings of the butterfly. It was hypothesized to be caused by effects associated with the environment, phenotypic plasticity or the signalling sources for position and morphology of pattern elements which only covers short distances.

Keywords: Fore wing, Hind wing, Modularity, Integration, *Melanitis leda*.

INTRODUCTION

The fore and hind wings of butterflies are serially homologous structures (Monteiro, 2008) subdivided into several compartments by a system of netted veins. In the common evening brown *Melanitis leda* (Linnaeus, 1758), compartmentalization in the wings bordered by veins are observable (Fig. 1). Each compartment seems to constitute a semi-independent developmental module, leading to prediction about constraints on the evolution of the wing. Studies suggest that wings having such compartments could be pondered as autonomous units of morphological variations and therefore can be seen as a separate developmental module (Garcia-Bellido and de Celis, 1992; Lawrence, 1992; Sturtevant and Bier, 1995; Klingenberg *et al.*, 2001; Torres *et al.*, 2010; Beldade *et al.*, 2002). Modularity describes the general idea that biological system at many hierarchical levels are not uniformly but it is rather about the differences in the degree of integration of part within and between sets of traits (Klingenberg, 2008). Modules are integrated tightly because there are many and often strong interactions among them, but different modules are relatively independent of each other because the interactions between modules are fewer or weaker (Klingenberg, 2008). Integration within each module is defined as the cohesion among traits that results from interaction of biological process producing the phenotypic structures (Klingenberg, 2008). Therefore, inferences about the boundaries of modules from the patterns of covariation among traits can be made by partitioning the traits into subsets in different ways and comparing the degree of covariation between subset (Klingenberg, 2008). Integration and Modularity is concern to the degree of covariation between parts of a structure, it can be studied by means of morphometric methods. It is assigned to determine whether the structure is a single

integrated unit or consists of several distinct units, and to identify the modules provided (Klingenberg, 2009).

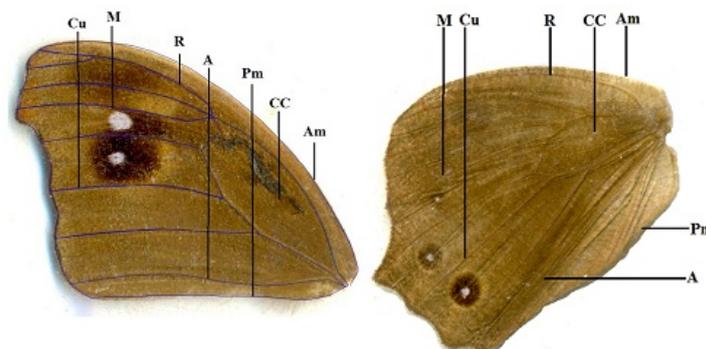


Fig.1: Wing venation pattern of the butterfly *M. leda* (Linnaeus) showing the major veins of A) fore- and B) hind wings: Am = Anterior margin, C = costa, R = radius, branched, M = Media, Cu = Cubitus, A = Anal vein, Pm = Posterior margin.

In this study, several hypotheses of morphological integration within the fore and hind wings of *M. leda* were tested. In particular, whether the compartments shared tight correlations between constituent compartments (i.e., highly integrated) or is more modularly organized (i.e., covariation is greater within regions than between) were tested. This was done using Modularity and Integration (MINT) analysis tool (Marquez, 2008). 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 (Marquez, 2008).

MATERIAL AND METHODS

Collecting and preparation of samples. Butterflies were collected using a lightweight, long handled sweep net. The collected butterflies were then placed delicately in a white paper envelope to prevent damage. 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 coloured, 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 transparent tape and then labelled. The mounted wings were scanned using a Hewlett-Packard Jacket 2400 scanner in a 1200 dpi resolution. The image was then cropped, sorted according to wing type and orientation and then saved.

Model Construction and Model Testing. A total of 195 points was used for outlining the shape of wings as well as their major vein pattern. TPSDig2 software (Rohlf, 2006) was used in digitally outlining the fore and hind wings. The outlined data was then converted to landmark points (XY) using TPS util (Rohlf, 2009) and then loaded to MINT (Modularity and Integration Analysis Tool). Different hypotheses (Table 1) were formulated to test and determine whether the entire wing of *M. leda* is a single module or whether the compartments are independent units.

Table 1: Developmental modules of the fore wing based on the wing venation pattern of *M. leda*.

MODEL	MODULES (No.)	DESCRIPTION
Forewing		
1	no modules	Null Model, there is no compartmentalization within the wings
2	1	every compartment serves as one
3	6	First Module bounded by the anterior margin and the 1 st radial vein Second Module bounded by the 1 st radial vein and the 1 st cubitus vein Third Module bounded by the 1 st cubitus vein and the 2 nd cubitus vein Fourth Module bounded by the 2 nd cubitus and the anal vein Fifth Module is the central compartment Sixth Module bounded by the anal vein and the posterior margin of the wing
4	5	First Module bounded by the anterior margin of the wings and the 1 st radial vein Second Module bounded by the 1 st radial vein and the 2 nd cubitus vein Third Module bounded by the 2 nd cubitus and the anal vein Fourth is the central compartment Fifth Module bounded by the anal vein and the posterior margin of the wing
5	3	First Module bounded by the anterior margin of the wings and the first radial vein Second Module bounded by the first radial vein and the anal vein (included the central compartment) Third Module bounded by the anal vein and the posterior margin of the wing
Hindwing		
1	no modules	Null Model, there is no compartmentalization within the wings
2	1	every compartment serves as one module
3	4	First Module bounded by the anterior margin and 2 nd radial vein Second Module bounded by the 2 nd radial vein and the 2 nd cubitus vein the central compartment Third Module bounded by the 2 nd cubitus vein and the posterior margin of the wing
4	3	First Module bounded by the anterior margin and the 2 nd radial vein Second Module bounded by the 2 nd radial and the 2 nd cubitus vein (central compartment) Third Module bounded by the 2 nd radial and the posterior margin of the wing
5	5	First Module bounded by the anterior margin and the 1 st radial vein Second Module bounded by the 1 st radial and the 2 nd radial vein Third Module bounded by the 2 nd radial and the 2 nd cubitus vein (central compartment) Fourth Module bounded by the 2 nd cubitus vein and the anal vein Fifth Module bounded by the anal vein and the posterior margin of the wing

Modularity and Integration Tool (MINT) for Morphometric Data version 1.5 (Marquez, 2008) was used to study modularity and integration in butterfly fore- and hindwings. *A priori* models were constructed using the model building option tool of the software. The process generated a total of 10 models of variational modularity in the shape data, including the null model that assumes that no modularity exist (Fig. 2). Each of these modules consists of a unit that is tightly integrated internally but relatively independent from the other modules (Torres *et al.*, 2010). 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 structures (Marquez, 2008).

The goodness of fit (GoF) tests, were employed to assess whether the hypothesis will be good enough to explain variation in a dataset. Resulting P-values and γ^* 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 γ^* values. This indicates a high degree of similarity between the proposed model and the observed data and thus the proposed model is accepted (Marquez, 2008).

RESULT AND DISCUSSION

Results of Modularity and Integration analysis are presented in Table (2). The top 3 best fit models for both the fore and hind wings of *M. leda* is supported by the standardized gamma values (g^* value) and P-values. According to Marquez (2008), P-values greater than 0.05 ($P > 0.05$) and approaching to 1 correspond to low γ^* -values

indicating a high degree of similarity between the proposed model and the observed data and thus the proposed model is accepted. The best fit models for the fore- and hindwings are shown in Table (2) and Fig. (2). The result is supported by the Gamma values obtained. The P-value of 1.0 for the null hypothesis that the data are no more different from this model than expected by chance is based on Wishart/Monte Carlo test with 1000 replicates.

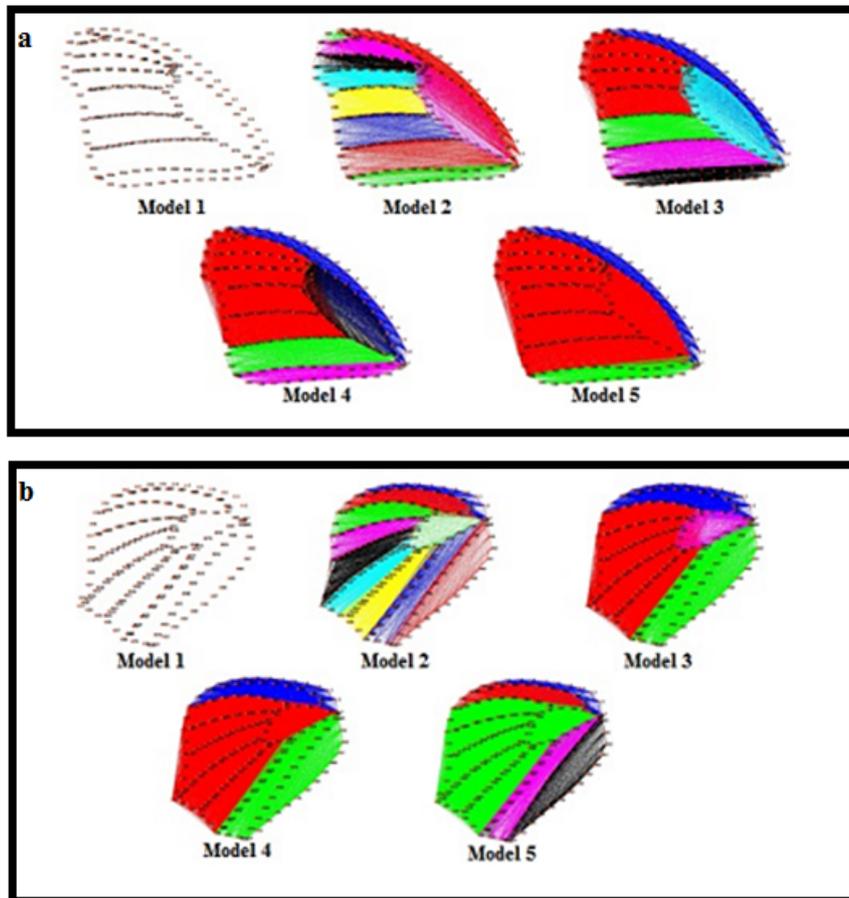


Fig. 2: Models used in this study for the fore- and hind wings of Common Evening Brown Butterfly, *M. Leda*.

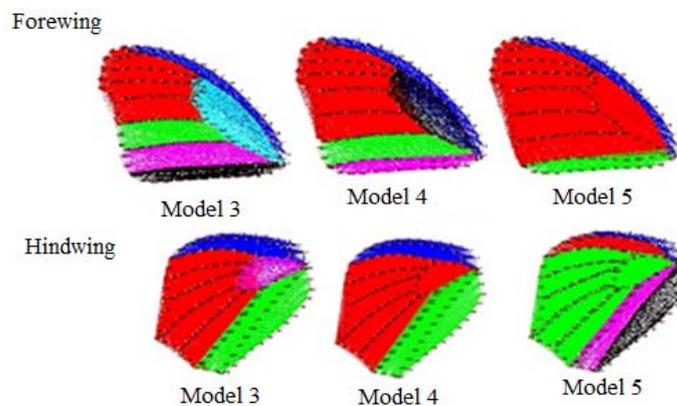


Fig. 3: Best model of fore and hind wings of *M. leda*.

Table 2: Top three best fit models for the left and right Fore- and Hind wings of male and female *M. leda*.

Forewing									
Female					Male				
Wing	Model	Rank	Gamma Value	P - Value	Wing	Model	Rank	Gamma Value	P - Value
Left	4	1	0.14784	1	Left	3	1	0.24945	1
	3	2	0.15386	1		4	2	0.25273	1
	5	3	0.21648	1		5	3	0.28287	1
Right	3	1	0.21187	1	Right	5	1	0.30567	1
	4	2	0.22319	1		4	2	0.32473	1
	5	3	0.27903	1		3	3	0.4245	0.599

Hindwing									
Female					Male				
Wing	Model	Rank	Gamma Value	P - Value	Wing	Model	Rank	Gamma Value	P - Value
Left	5	1	0.17487	1	Left	3	1	0.2535	1
	4	2	0.18619	1		5	2	0.2566	1
	3	3	0.18743	1		4	3	0.25962	1
Right	5	1	0.2092	1	Right	5	1	0.3891	1
	4	2	0.21631	1		4	2	0.39062	1
	3	3	0.21776	1		3	3	0.3996	1

The results in Table (2) and Fig.(3) show that the top three best fit models of the left and right fore wings of the male and female are on different ranks. Within the female sex, the left and right hind wings showed the same ranks of the best fit models while the male differences were different. Although the ranks are different, the top 3 models can be considered similar as shown by very minute variation in the gamma values obtained. These minor differences in the best fit results could be explained by factors of modularity.

It might be due to the effects associated with the environment, phenotypic plasticity) or factors that act during onto-genetic development (Mutanen and Kaitata, 2006; Meyer-Rochow and Lau, 2008; Sihvonen, 2008; Benitez *et al.*, 2011). The differences in the number of modules shown by the top 3 models could have a developmental basis. Lack of internal constraint is plausible where the position and morphology of each pattern element determined by signalling sources have effects extending only over short distances since the signal does not appear to pass across the wing veins (Nijhout, 1994) or there were lack of physical communication between them and/or from the wing-cell-specific genetic composition (Beldade and Brakefield, 2003). The results in this study, however, are in conformity to a number of studies suggesting that insect wings are divided into compartments each of which is a separate developmental module (Cavicchi *et al.*, 1991; Zimmerman *et al.*, 2000; Klingenberg *et al.*, 2001; Torres *et al.*, 2010; Tabugo *et al.*, 2011) and that these compartments may assimilate to distinct cell lineages and domains of gene expression (Garcia-Bellido *et al.*, 1973; Lawrence, 1992).

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