

Hybridization in the species of *Enantia jethys* complex (Lepidoptera, Pieridae)

Jovana M Jasso-Martínez, Salima C Machkour-M'Rabet, Roger Vila, Rosario Rodríguez-Arnaiz, América N Castaneda-Sortibrán

Abstract

With at least 10% of the worldwide species involved, hybridization is common in animal species. Hybridization events have been largely demonstrated in butterfly natural populations. Studies have shown that interspecific gene flow remains important even after speciation, and therefore hybridization and introgression are an important factor for the evolution of species as a source of genetic variability. *Enantia* is a butterfly genus of the family Pieridae, and currently contains nine Neotropical species. The *Enantia jethys* complex is a Mesoamerican group composed by three species (*E. jethys*, *E. mazai*, *E. albania*), all of which are sympatric in Mexico. Results: We carried out separate and concatenated phylogenetic analyses among Mexican specimens of the above taxa using DNA sequences of three gene markers (COI, RpS5, Wg) and ISSRs. The separate analyses recovered distinct topologies and all markers had high levels of interspecific gene flow. We found evidence of directional hybridization. Hybridization always involves *E. albania* with the other two species, but it never occurs between *E. jethys* and *E. mazai*. We also observed that the hybrids can affect the levels of genetic diversity in these species. This study remarks the importance of assessing the presence of hybridization in evolutionary studies of closely related species.

Introduction

Natural hybridization can be defined as the production of viable hybrids from interspecific mating, and introgression as the integration of foreign genetic material from one species into another through backcrossing (Baack and Rieseberg 2007). Hybridization process is common in animal species and it have been largely demonstrated in natural butterfly populations. The *Enantia jethys* complex is a Mesoamerican group composed by three species (*E. jethys*, *E. mazai* and *E. albania*) in sympatry in Mexico (Llorente-Bousquets, 1984; Jasso-Martínez et al., 2016). The existence of hybridization in this group is supported by field expeditions that have permitted the observation of separate events of interspecific mating.

Methods

We employed sequence molecular markers –mitochondrial and nuclear– and additionally we used Inter Simple Sequence Repeats markers (ISSRs) to examine contemporary genetic exchange among the butterflies of the *Enantia jethys* complex. A total of 339 individuals of the three species from sympatric populations were collected in Veracruz and Puebla (Mexico). We measure the levels of genetic diversity in each species and we performed an STRUCTURE analysis. With a sub-dataset (83 individuals) we carried out separate and concatenated phylogenetic analyses using DNA sequences of three gene markers (COI, RpS5, Wg).

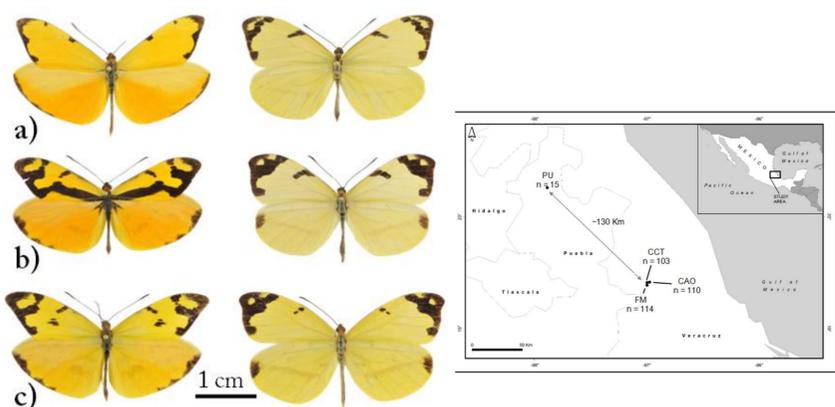


Figure 1. Left: The species of *Enantia jethys* complex a) *Enantia albania* Bates, 1864, b) *E. jethys* Boisduval, 1836 and c) *E. mazai* Llorente, 1984. Right: Map of taxonomic sampling in Mexico.

Results

Through STRUCTURE analysis we found three general genetic components which correspond to the three species in the complex. Additionally, we found individuals with genetic components from two species (hybrids) (Figure 2). The species of *Enantia jethys* complex have high levels of polymorphism and genetic diversity (Table 1). We did not observed significant change in the level of genetic diversity between hybrid and non-hybrid individuals within *E. albania*. In *E. jethys* we observed lower values of genetic diversity in hybrid individuals than in non-hybrids. On the other hand, in *E. mazai* we found higher values of genetic diversity in hybrid individuals versus non-hybrids. We obtained three different topologies through the sequence markers, but RpS5, COI and our concatenated analysis show three clades which corresponds with the three species previously described.

References

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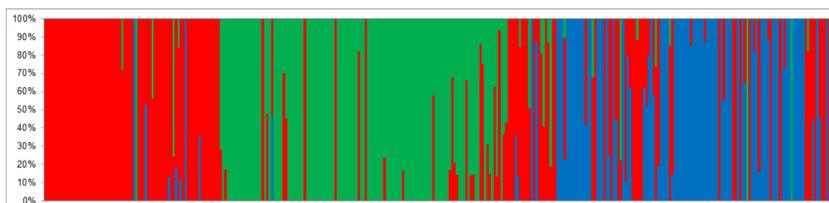


Figure 2. STRUCTURE analysis. Each color is representing a genetic component through ISSRs: red = *E. albania*, green = *E. jethys* and blue = *E. mazai*.

	<i>E. albania</i>			<i>E. jethys</i>			<i>E. mazai</i>		
	Total n = 76	Non-hybrids n = 63	Hybrids n = 13	Total n = 123	Non-hybrids n = 93	Hybrids n = 30	Total n = 140	Non-hybrids n = 66	Hybrids n = 74
TB	64	62	55	63	63	59	61	42	61
RB	6	5	0	3	3	4	14	16	8
PB	1	2	1	2	3	1	0	0	0
%P	97	94	83	95	95	89	92	64	92
$h \pm SD$	0.195 ± 0.13 ^a	0.193 ± 0.13	0.185 ± 0.13	0.254 ± 0.14 ^a	0.263 ± 0.14	0.207 ± 0.15	0.136 ± 0.11 ^c	0.071 ± 0.01	0.188 ± 0.14
		Ns			*			***	

Table 1. Levels of polymorphism and genetic diversity in each species of the *Enantia jethys* complex, and in each portion of hybrids and non-hybrid individuals.

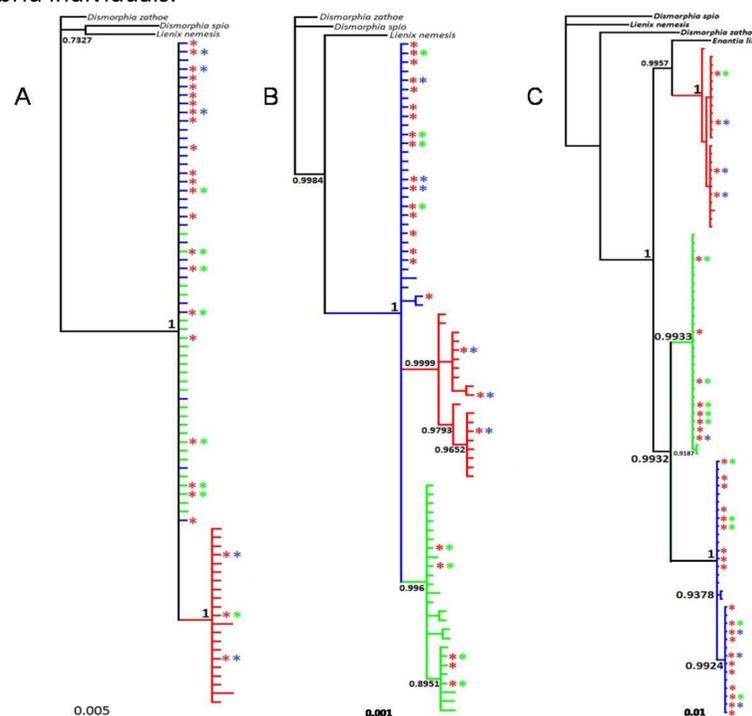


Figure 3. Trees through molecular markers. a) Wg, b) RpS5 and c) COI.

Discussion

The maintenance of high polymorphism levels have been observed in species with high population density in restricted geographic areas (*Baronia brevicornis*, Machkour-M'Rabet et al., 2014). Observation in the field lead to us suggesting high population density in this species. We observe hybridization patterns with ISSRs but it did not observed through sequence markers. Sequence markers used correspond to specific loci in the genome, while ISSR sampling randomly in the genome. It is possible that the sites sampling by ISSRs correspond with sites that hybridize faster than others, showing contemporary hybridization events among the species of the complex. The directional hybridization pattern observed could be suggesting some reproductive barriers among species of the complex and difference in the viability of hybrids. COI marker is very useful in the delimitation of species as well our results shows.

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