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Biological Research at Butterfly Farms in Costa Rica

In a recent trip to Costa Rica, Antónia Monteiro, assistant professor in the Yale Department of Ecology and Evolutionary Biology (EEB) and assistant curator of entomology at the Peabody Museum of Natural History (YPM), and Xiaoling Tong, Postdoctoral Associate, worked closely with butterfly farmers to collect larval samples at particular developmental stages from a variety of different species.

The two Yale biologists visited the oldest butterfly farm in Costa Rica, Suministros Entomologicos Costarricenses (*www.butterflyfarm.co.cr*), an exporting center for live pupae reared in the region's surrounding farms. The purpose of the trip was to sample larval wings from 20 different species of butterflies to later examine the expression of genes involved in the color patterning of the adult wings. Because the farm only exports live pupae, and the Yale biologists needed to examine larval patterns of gene expression, they arranged to collect the larvae directly from the farms, before they metamorphosed into pupae.

The analysis of gene expression in larval wings is part of a National Science Foundation-funded project, awarded to Monteiro and William Piel, YPM associate director of bioinformatics, with the goal of understanding how eyespot patterns evolved and diversified in Nymphalid butterflies. This group of butterflies includes the spectacular blue morphos, the crackers, and the owl butterflies, all displaying variable numbers of eyespots on their wings. One of the aims of the project is to examine to what extent wing spots of single color and eyespots, with concentric circles, express the same genes and represent variations of the same patterning process. Another goal is to test whether eyespot number diversity is accomplished by deleting eyespots from a

A *Morpho peleides*

B *Saturniid larve*

C *Colobura dirce*

putatively ancestral species with many eyespots, or, instead by adding eyespots to novel wing compartments over time.

Butterfly houses around the world import most live pupae from butterfly farms located in the tropics, and farms in Costa Rica are some of the oldest suppliers. Monteiro and Tong visited two of the largest farms in the area. Costa Rica is home to some 550 species of butterflies, and roughly 100 of these are farmed for export. The right food plants are grown in small fields, then enclosed in large net enclosures, and the adults released inside. The butterflies lay eggs and the larvae eat the food plants protected from most parasitoids and predators. Once a field of plants is devoured, no more adults are allowed inside until the plants recover. A different field is then used for growing the larvae. This system of field rotations resembles how larger herbivores, such as cows and sheep, are reared in traditional farms. Once the larvae reach the last larval stage they are taken indoors and fed cut plants, for closer monitoring of development. The pupae are harvested one or two days after pupation and sent to the Suministros for export. Roughly 80,000 to 120,000 pupae, provided by 150 different local farms, are shipped to butterfly houses in the United States and Europe every month.

Butterfly farmers, perhaps without realizing it, have much to contribute to basic biological research in the field of the evolution of development. The farms rear the same species year around and function as a stock center for butterfly biodiversity. But, more importantly, given the difficulty of rearing some of these tropical species in more northern latitudes, the farms provide a source of valuable biological material that can be sampled in large quantities, at any time, and at any stage of development. These farms are, thus, fantastic resources for researchers that are interested in delving into comparative aspects of developmental biology using butterflies as model species.

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D *Dryadula phaetusa (Nymphalidac)*

E *Hymadras arinome*

F *Archaeoprepona demophoon*

G *Hymadras februa*

H *Morpho peleides (Nymphaelidae)*

I *Tithorea tarricina*