Final Summary Report

Project Title: Nutritional Ecology and Seasonal Color Polymorphism in a Butterfly

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Overview

Over the past 100 years, considerable attention has been paid to the physiological mechanisms underlying alternative seasonal color morphs in butterflies, a phenomenon more generally described as seasonal color polyphenism. This past research largely focused on the endocrine control of color phenotype, and its interaction with seasonal changes in photoperiod and temperature. However, recent work has highlighted the material costs imposed by color patterns, particularly for colors whose basic constituents must be derived from diet (e.g. carotenoid coloration in birds). Essentially nothing is known about the costs of seasonally polyphenic color patterns in butterflies, how these correspond to seasonal shifts in nutrient availability, or how such costs are integrated into the seasonal life history strategies of these colorful animals. However, nearly all butterfly colors are synthesized from essential amino acids, suggesting that they compete directly with other traits for these important dietary constituents.

The goal of this EU Marie Curie IIF project was to consider the material costs of distinct seasonal color phenotypes within the eco-evolutionary context of 1) seasonal changes in nutrient availability and 2) seasonal differences in life history strategy. We selected the seasonally polyphenic European Map Butterfly, *Araschnia levana*, as the focal organism for this work, in part because much is known about the natural history and hormonal control of seasonal polyphenism in this butterfly. In France, this butterfly has three generations a year. During the summer months, two consecutive generations develop directly from egg to adult, producing adult butterflies with a striking black-and-white, melanin-based wing patterning (see Fig. 1). These "summer form" generations are followed by a third generation that develops from egg to pupae during the fall months, overwinters in a pupal diapause, and emerges in mid-spring as "spring form" adults with bright orange, ommochrome-based wing patterning (see Fig. 1). Importantly, these two different color forms require large quantities of two distinct essential amino acids: tyrosine for the eumelanin in "summer form" adults and tryptophan for ommochrome pigmentation in "spring form" adults.

Experimental Approach

Our experimental design consisted of three major studies: 1) the characterization of naturally-occurring, seasonal nutrient dynamics, both in the host plant, *Urtica dioica*, and the pupal and adult tissues of *A. levana*; 2) hormonal manipulation of the developmental trajectories of "spring form" and "summer form" individuals to elucidate functional tradeoffs between color phenotype and other life history characters (e.g. fecundity), and 3) pupal injections of radiolabeled pigment precursors to more precisely identify tissues in direct and/or indirect resource competition with each color phenotype.

Figure 1. "Spring form" (left) and "summer form" (right) phenotypes of *A. levana.*

Major Findings

The nutrient content of host plant tissues plays a fundamental role in the resources available to herbivores during development, and thus dictates the costs and benefits of particular traits. *A. levana* feeds exclusively on the stinging nettle, *Urtica dioica*. Seasonal sampling of host plant tissues for nutritional content (water, protein, lipids, and sugars) revealed that the nutritional value of *U. dioica* degrades over the course of the summer-fall growing season. Fall leaves of this host plant are lower in lipids, sugars and water content, although equivalent in bulk protein. This suggests that "spring form" *A. levana* adults, which feed as larvae during the fall months, experience a more nutritionally challenging environment during development. Consistent with this pattern of host plant composition, "spring form" pupae were found to be smaller and have lower water content, even when reared on the same diet as "summer form" pupae. Together, these results hint at an adaptive tuning of seasonal nutrient requirements to match patterns of seasonal nutrient availability. We are currently analyzing data to understand how these differences in resource availability and acquisition influence patterns of adult resource investment in relation to the two color phenotypes.

Based on the results above, we used hormonal manipulations to "force" individuals prepared for one developmental trajectory to switch developmental fates and produce the alternative form (e.g. "spring form" pupae manipulated hormonally to produce "summer form" adults). Due to high mortality within some of our experimental treatments, data collection on this large study has just been completed. Thus, data analysis is currently underway to fully appreciate the costs and tradeoffs observable between the focal color traits and other life history characters. However, one notable result has emerged. Females whose developmental fate was switched with a hormone injection were in most cases able to produce the alternative color form, but these females exhibit a 2-5 fold reduction in fecundity. This suggests that specific costs associated with producing each color form do exist, but that such costs are largely met by the nutrient acquisition strategies of developing larvae. When an experimentally-induced mismatch occurs between the costs of a phenotype and the resources acquired to meet those costs, significant fitness reductions occur. This result is an elegant demonstration of a theoretical concept central to life history theory.

Lastly, we used radiolabeled amino acids to identify the pattern of resource competition between coloration and other traits during pupal development. Preliminary results from this ongoing study indicate that coloration in this species competes with tissues throughout the body for the amino acids that serve as pigment precursors. This suggests that both color phenotypes in the butterfly species are likely to interact strongly with other traits when resources are limiting, consistent with the fecundity costs noted above.

Future Directions

The research program conceived and executed during the Marie Curie fellowship was designed to address questions related to the current costs and benefits of the two seasonal color forms in this butterfly. However, questions still remain regarding the origin of this seasonal polyphenism. What initiated and drove the evolutionary shift from monomorphism to seasonal polyphenism? The best evidence to date suggests that seasonal polyphenism in this group likely originated in an ancestral species living in what is modern-day China. Pilot studies we conducted during the final months of the fellowship suggest one possible mechanism that may have been involved in the origin of this seasonal polyphenism. In particular, preliminary data hint that precursors in the ommochrome synthetic pathway may actually play a direct functional role in the hormonal control of developmental timing. Thus, historical selection on developmental timing may have resulted in concurrent shifts in coloration, resulting in two distinct color forms. This exciting possibility will be the subject of future collaborative efforts between the labs of J. Casas (Université de Tours) and N. Morehouse (now assistant professor, University of Pittsburgh).