Butterfly wing scale shape: phylogenetic relationships or convergent evolution?

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Introduction

Setae modified into broad, flat scales are found in several insect orders, but it is these scales that are characteristic of the wings of Lepidoptera (butterflies and moths). Lepidopteran scales are arranged like roof shingles in rows that run anterior to posterior across the wing. Each scale displays only one color; it is the combination and arrangement of different colored scales that create the elaborate patterns seen on many butterfly wings. The colors and patterns that scales create are involved in thermoregulation, sexual selection, signaling, camouflage (crypsis), warning colors (aposematism), and mimicry. Scale color can be caused by pigment, such as in blacks, browns, reds, whites, and yellows, but iridescent colors, like violets, greens, and golds cannot be produced by any known pigments. These colors are produced by nanoscale interactions between light and complex cuticular structures of the scale. Each scale is a single cell consisting of a stalk and a hollow blade. Longitudinal ridges run along the surface of the blade and cross ribs lie between and perpendicular to each ridge. Basic structures of the scale can be elaborated upon to produce specific reflective structures, e.g., the longitudinal ridges or cross ribs can be fringed with lamellae or the lumen can contain stacks or lattice structures to produce interference reflection or diffraction to create color.

I hypothesized that scales with similar shapes and properties would be found either among closely related species of butterflies or species that have wings of a similar color. Viceroy (Linantis archippus, Cramer 1776) and Red-Spotted Purple (Linantis arthemis astyanax, Fabricius 1775) butterflies are closely related and produce the same color wild. Viceroy and Monarch (Danaus plexippus) butterflies are Müllerian mimics, thus they share a similar coloration. By examining the shape of scales from each species we can determine if scale shape is a result of phylogenetic relationships or convergent evolution.

Materials and Methods

Hind wings from three nymphalid butterflies (D. plexippus; L. archippus; and L. a. astyanax, n=3 each; Figure 1A) were obtained from dead specimens. The dorsolateral sides of hind wings from each species were imaged with an Olympus Tough TG-860 digital camera on the macro setting (Figure 1B) and a Dino-Lite Edge AM4515ST digital microscope (Figure 1C). The discal cell of each wing was dissected out and cut into three sections: proximal, medial, and distal. Different sections from each individual were placed together to form a whole discal cell composed of samples from three individuals, which was done for all species. The composite discal cells were then placed on an aluminum scanning electron microscopy (SEM) stub and were sputtercoated with 7 nm of platinum. The discal cells were then imaged at 15 kV, 500X magnification with a JEOL 6010LA SEM. Scales from a proximal, medial, and distal portion of the discal cell of each species were imaged.

Scale length, scale width, protrusion width, cross rib width, and scale margin width (Figure 2A) were measured with ImageJ software (http://image.j.nih.gov/ij/) from three scales from each SEM image (3 images per species). The number of protrusions for each scale were counted and the shape of the scale was classified as smooth, scalloped, wavy, saw-toothed, or abnormal (Figure 2B). Analysis of variance (ANOVA) with a Student’s T post-hoc test was performed in JMP 13 for each measurement (scale type was converted to numbers 1 through 5) by species to determine significant differences (p<0.05).

Results

ANOVA revealed significant differences by species for scale length, scale width, protrusion width, margin width and number of protrusions (Figure 3). There were no significant differences in cross rib width or scale type. Danaus plexippus and L. archippus differed significantly in scale length (p<0.0161) and width (p=0.0113) while L. a. astyanax did not differ significantly from either species. Limenitis arthemis astyanax and D. plexippus had significant differences in projection width (p=0.0276) and number of projections (p=0.0379) while L. archippus did not differ significantly from D. plexippus or L. a. astyanax for either measurement. Both L. archippus and L. a. astyanax differed significantly from D. plexippus in scale margin width (p<0.0173 and p<0.0037, respectively) but did not differ from one another. Although there were not significant differences in scale type, L. archippus and L. a. astyanax had the highest number of scale types (smooth, scalloped, wavy and saw-toothed) while D. plexippus only showed smooth, wavy, and abnormal scales.

Discussion

For every scale dimension measured, L. archippus and L. a. astyanax were not statistically different from one another. Additionally, either L. archippus or L. a. astyanax (or both species of Limenitis) differed significantly from D. plexippus in all measurements where significant differences were found. These results indicate that similarity in scale shape is likely due to phylogenetic relatedness rather than convergence in scale coloration. However, the cuticular architecture that creates structurally-induced colors is nano-sized and can be located within the scale’s lumen, thus structures responsible for colors as iridescent blue would not be visible with these methods. Additional studies at the nanoscale, where both pigments and light refracting and diffracting structures would be visible, might yield results indicating convergence in similarly colored scales.

Figure 1. A) Red-spotted purple (L. a. astyanax), Viceroy (L. archippus), and Monarch (D. plexippus) butterflies (top to bottom). B) Macro photography images of hind wings of each species. C) Digital microscopy images of hind wing scales. D) SEM images of scales at 500X magnification used for measurements.

Figure 2. A) Measurements made for each scale included length (L), width (W), number of projections, protrusion width (PW), cross rib width (CRW) and scale margin width (SMW). B) Scales were classified as smooth, scalloped, wavy, saw-toothed, or abnormal (top to bottom).

Figure 3. ANOVA with Student’s T test revealed significant differences (indicated with A and B) in scale length, scale width, projection width, number of projections, and scale margin width between D. plexippus, L. archippus, and/or L. a. astyanax.

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