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REVIEW

CENTENARY ARTICLE

A meta-analysis of butterfly structural colors: their color range, distribution and biological production

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ABSTRACT

Butterfly scales are among the richest natural sources of optical nanostructures, which produce structural color and iridescence. Several recurring nanostructure types have been described, such as ridge multilayers, gyroids and lower lamina thin films. While the optical mechanisms of these nanostructure classes are known, their phylogenetic distributions and functional ranges have not been described in detail. In this Review, we examine a century of research on the biological production of structural colors, including their evolution, development and genetic regulation. We have also created a database of more than 300 optical nanostructures in butterflies and conducted a meta-analysis of the color range, abundance and phylogenetic distribution of each nanostructure class. Butterfly structural colors are ubiquitous in short wavelengths but extremely rare in long wavelengths, especially red. In particular, blue wavelengths (around 450 nm) occur in more clades and are produced by more kinds of nanostructures than other hues. Nanostructure categories differ in prevalence, phylogenetic distribution, color range and brightness. For example, lamina thin films are the least bright; perforated lumen multilayers occur most often but are almost entirely restricted to the family Lycaenidae; and 3D photonic crystals, including gyroids, have the narrowest wavelength range (from about 450 to 550 nm). We discuss the implications of these patterns in terms of nanostructure evolution, physical constraint and relationships to pigmentary color. Finally, we highlight opportunities for future research, such as analyses of subadult and Hesperid structural colors and the identification of genes that directly build the nanostructures, with relevance for biomimetic engineering.

KEY WORDS: Lepidoptera, Scale, Photonic nanostructure, Development, Genetics, Evolution

Introduction

Structural color occurs when light is scattered by nanoscale structures made of a dielectric material (see Glossary), causing constructive and destructive interference effects (see Glossary) that result in the pronounced reflection of specific wavelengths (the same principle on which soap bubble iridescence operates; see Glossary). A better understanding of biological photonic nanostructures (see Glossary), and the mechanisms that produce them in living systems, has recently been identified as a top research priority by physicists and biologists who investigate color and the manipulation of light (Cuthill et al., 2017; Wilts and Vignolini, 2018). Currently, butterflies are a leading system in this effort, with a recent surge of progress on the genetic and developmental processes that regulate diverse structural colors (see Glossary) (Zhang et al., 2017; Thayer et al., 2020; Brien et al., 2022; Ficarrotta et al., 2022; Prakash et al., 2022). Lepidopteran insects build morphologically diverse photonic nanostructures that result in optical effects such as iridescence, selective interactions with polarized light (Saba et al., 2014; Zhang et al., 2014), and dynamic color displays that change in response to humidity levels (Wilts et al., 2019). Butterfly structural colors also contribute to various ecological functions, including camouflage (Wilts et al., 2013), thermal regulation (Biró et al., 2003) and sexual signaling (White et al., 2015).

Butterfly optical nanostructures (see Glossary) are found as parts of scales, which are epithelial structures around 300 µm long that protrude from sockets on the wing membrane (Fig. 1A). Scales are non-living structures made of the polysaccharide chitin, each produced by one cell during pupal wing development (Stossberg, 1938). Evolutionarily, they are derived from sensory bristles like those found on fly wings (Galant et al., 1998). A typical scale is shaped like a flat tile, with a set of conserved sub-elements that are all made of chitin (defined by Ghiradella, 1984; Fig. 1A–C). The adwing surface (see Glossary) of the scale is a flat layer called the lamina, which spans the scale's full dimensions (purple, Fig. 1B,C). On the abwing surface (see Glossary) of the scale, rows of parallel ridges extend from the basal to the apical tip of the scale (red, Fig. 1B,C). These ridges have smaller subunits which are called ridge lamellae or scutes. Crossribs are oriented perpendicularly to the ridges in the same plane and connect pairs of ridges to form a grid (yellow, Fig. 1B,C). On their lateral sides, ridges bear microribs (green, Fig. 1B,C) that stretch from the ridge's apex to the height at which the crossribs join. The entire network of ridges and crossribs is held above the lamina by spacers called trabeculae (blue, Fig. 1B,C). The open space in the scale's interior is the lumen. There may be chemical pigments (e.g. melanin, ommochromes, papiliochromes; see Glossary) in any part of the scale (Stavenga et al., 2014a; Wilts et al., 2015). Because chitin has a sufficiently high refractive index (~1.5; Leertouwer et al., 2011; see Glossary), and because scales have many intricate parts in the appropriate size range to interact selectively with wavelengths in the visible spectrum, scales are an excellent substrate for structural color.

The fine structures and associated optical properties of structurally colored butterfly scales have been progressively elucidated over the last century. By the early 1900s, various scientists recognized that the iridescent colors of *Morpho*, *Papilio*, *Hypolimnas* and other butterfly genera were not pigmentary, but rather involved more complex interference effects (Michelson, 1911; Rayleigh, 1919; Onslow, 1923). Mason (1926, 1927) used combinations of light microscopy, chemical treatments and spectral observations under a battery of illumination conditions to correctly deduce the refractive index of chitin and several key aspects of *Urania* and *Morpho* scale morphology. However, the resolving

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Glossary

Adwing and abwing
In the scale's natural orientation, the adwing scale surface is nearer to the wing membrane, i.e. the lower lamina surface. The abwing surface faces outward from the wing, i.e. the ridge-bearing surface.

Bauplan
The generalized set of typical morphological features shared by a group of organisms.

Broadband reflector
Used here in a general sense to describe structures that reflect many wavelengths with similar intensities (i.e. structures that reflect a large bandwidth), as opposed to structures that produce reflectance spectra with a clearly discernible peak wavelength. Specifically, this describes the white, silver, pearlescent and some of the golden structural colors.

Color space
The range of possible colors that theoretically could be produced or visually discriminated.

Dielectric material
An insulating material that transmits electromagnetic waves without conducting electric charge.

Downstream genes
A downstream gene is relatively later acting in the genetic pathway, i.e. a set of genes that function in sequential steps of a biological process. In a regulatory genetic pathway, the activity of downstream genes may be directly controlled by upstream genes.

Interference
The result of an interaction between two electromagnetic waves; constructive interference creates a combined wave with greater amplitude if the waves are in phase; destructive interference creates a combined wave with lower amplitude if they are out of phase.

Iridescence
Shifting color depending upon the angles of illumination and/or observation.

Optical or photonic nanostructures
Structures whose dimensions are of the order of the wavelength of light, leading to reflection and interference effects when light interacts with the structure.

Photonic crystals
The subset of optical nanostructures that have a periodically repeating pattern, such as multilayer and gyroid structures in butterflies. These are further categorized as 1-, 2- or 3-dimensional according to the number of axes having a periodic pattern (see Biró and Vigneron, 2011).

Pigments
Chemical molecules that selectively absorb certain wavelengths of light. Examples in butterflies include melanin, ommochromes, papiliochromes, bilin and pterin pigments.

Plastic/plasticity
Trait variation in response to the environmental conditions.

Refractive index
A measure of the relative speed of light in different media. When light passes between media, differences in their refractive indices determine the angles of reflection and refraction.

Scatterometry
Here, a technique used to assess a photonic structure's reflective behavior across many angles (e.g. Stavenga et al., 2009).

Structural color
Color that results from the selective reflection of specific wavelengths of light as it interacts with optical nanostructures.

power of light microscopy is limited to features of the order of several hundred nanometers, so optical nanostructures could not be directly imaged, and their interpretation remained coarse. The advent of electron microscopy (EM) in the 1930s and 1940s provided a crucial new tool for observing nanostructures, and the first electron micrographs of butterfly scales were taken of *Morpho cypris* and *Morpho achilles* (Anderson and Richards, 1942; Gentil, 1942). EM technology gradually improved in subsequent decades, spurring on efforts to investigate nanostructure morphologies (Lippert and Gentil, 1959; Schmidt and Paulus, 1970; Huxley, 1975; Morris, 1975). Ghiradella's prolific work in the 1970s–1990s cataloged nanostructures from many taxa (e.g. Ghiradella, 2010), interrogated the processes of scale development (Ghiradella, 1974, 1989) and described both the scale Bauplan (see Glossary) and the classes of morphological departures from it which produce structural color (Ghiradella, 1985, 1998). Since the 1990s, an accelerating number of studies have characterized photonic structures with increasing methodological sophistication from a growing number of butterfly species (Biró and Vigneron, 2011).

In aggregate, the past century of research on butterfly structural color has shown that any part of a scale can function as an optical nanostructure, given appropriate evolved modifications. Previous reviews have categorized butterfly structures in terms of their morphology and associated optical mechanism (Srinivasarao, 1999; Ingram and Parker, 2008; Kinoshita et al., 2008; Ghiradella, 2010; Biró and Vigneron, 2011; Zhang et al., 2015; Mouchet and Vukusic, 2018). These categories are summarized in Fig. 1D–K, from top to bottom of the scale. In many species, elongated ridge lamellae overlap each other to form multilayers at the top of the scale (Fig. 1D) (Kinoshita et al., 2002; Wilts et al., 2011; Han et al., 2012). This characteristic morphology has been called 'ridge iridescence' or 'Christmas-tree structures,' while scales that bear such ridge structures are often termed '*Morpho*-type' scales. For a similar effect, other scales have tilted or enlarged microribs, such that the microribs overlap to form a multilayer (Fig. 1E) (Ghiradella, 1985; Wickham et al., 2006; Wilts et al., 2016). Crossribs may be expanded and fused with the microribs to form a solid upper surface, creating a bilayer system that also involves the lower lamina (Fig. 1F) (Simonsen, 2007; Vukusic et al., 2009). In a related bilayer morphology, crossribs are expanded into vertical air columns that form a light-diffusing upper layer (Fig. 1G) (Trzeciak et al., 2012). In some Pierid butterflies, pterin pigments are clustered into granules that are suspended in the lumen of the scale (Fig. 1H) and cause incoherent light scattering (Stavenga et al., 2004; Morehouse et al., 2007). Three general categories of structures occur inside the scale lumen: continuous multilayers (Fig. 1K), perforated multilayers (Fig. 1I) and 3D photonic crystals (Fig. 1J; see Glossary). Multiple continuous layers in the lumen form a multilayer in *Papilio* and other butterflies (Fig. 1K) (Vukusic et al., 2000). Scales with lumen multilayers have often been called '*Urania*-type' scales after the first-reported lumen multilayers in sunset moth scales (since reclassified as *Chrysiridia*; Lippert and Gentil, 1959; Yoshioka and Kinoshita, 2007). Perforated multilayers, also called 'pepper-pot' structures, are common in Lycaenid butterflies (Wilts et al., 2009; Bálint et al., 2012) (Fig. 1I). Lastly, the lumens of certain scales are full of highly derived 3D photonic crystals, including inverse opals and gyroids (Michielsen and Stavenga, 2008) (Fig. 1J). At the bottom of the scale, a standard lamina can act as a simple thin film reflector (Fig. 1B) (Stavenga et al., 2014a; Thayer et al., 2020). Nearly all known butterfly structural colors belong to one of these categories.

While butterfly structural colors have been categorized according to their morphology, the biological differences among the categories are less well understood. For example, what is the phylogenetic distribution of each kind of structure? Which structures and colors are most and least common, and why? Is there evidence for taxonomic specificity or convergent evolution? Are there functional distinctions among the categories, such as differences in the ranges of color or brightness produced? To address these questions, we conducted a meta-analysis of the

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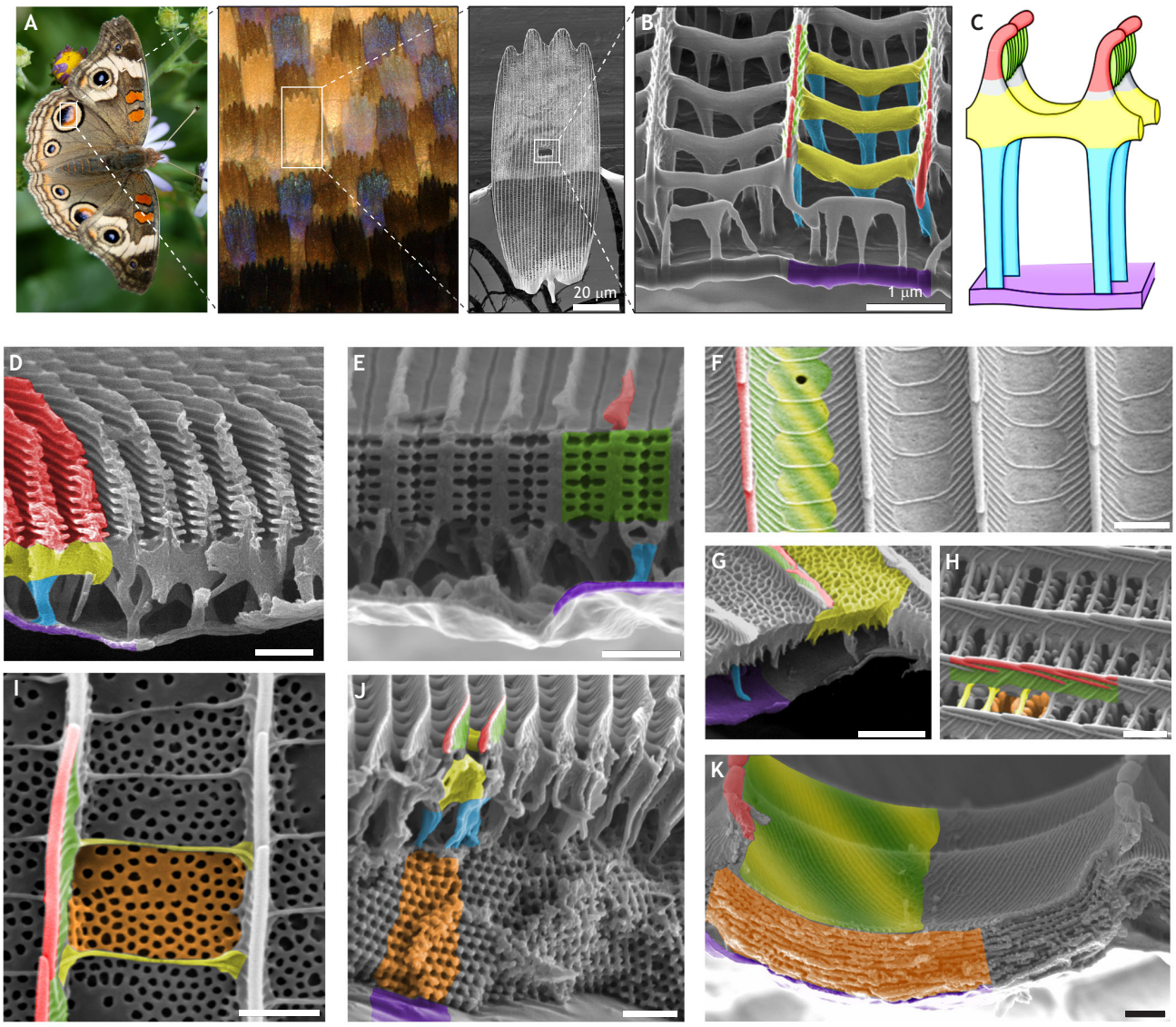

Fig. 1. Derived morphologies in butterfly scales cause structural color. (A) A standard butterfly scale (from *Junonia coenia*) and its parts in relation to the wing. Adapted from Thayer et al. (2020). (B–K) Overlaid colors label the homologous sub-elements of the scale Bauplan: red, ridges; green, microribs; yellow, crossribs; blue, trabeculae; violet, lamina; orange, novel structures with unknown homology; mixed colors indicate likely contributions of multiple sub-elements to a derived structure, and the lumen is the open space in the middle of the scale. (B) Cross-section of a standard scale (*J. coenia* from A; Thayer et al., 2020) shows its single layer lamina, which can cause thin film structural color. (C) Color-coded cartoon of scale sub-elements. Illustration credit: Dennis Sun. (D) Cross-sectioned blue *Morpho cypris* scale with modified ridge lamellae. Image credit: Ryan Null. (E) Cross-sectioned green *Trogonoptera brookiana* scale with modified microribs. Image credit: Aster Taylor. (F) Silver *Agraulis vanillae* scale with filled windows (top view). Image credit: Ryan Null. (G) Columnar crossribs in a blue *Papilio bromius* scale (cross-section). Adapted with permission from Trzeciak et al. (2012; ©The Optical Society). (H) White *Colias eurytheme* scale with pigment granules (top view). Image credit: Anna Czarkwiani. (I) Perforated multilayer in the lumen of a blue *Narathura japonica* scale (top view). Image credit: Bodo Wilts. (J) Green *Parides sesostris* scale with a crystal structure in the lumen (cross-sectional view). Image credit: Aster Taylor. (K) Cross-section of a green *Papilio palinurus* scale with a multilayer in the lumen. Image credit: Aster Taylor. Scale bar (B–K): 1 µm.

aggregated reflectance and morphology data for more than 300 previously reported butterfly optical nanostructures. We found that color range, brightness and phylogenetic distribution differ among the several morphological categories of optical nanostructures. These patterns have implications for the functionality and evolution of butterfly nanostructures and provide a reference against which the novelty of future structure descriptions can be judged. Below, we also review what is known about the biological processes that generate structural colors, including developmental events, genetic regulation and evolution. Throughout, we highlight outstanding questions and understudied taxa as suggestions for future research.

Meta-analysis methods

We used several intersecting approaches to search for articles, book chapters and theses reporting nanostructures that produce structural colors in butterflies. First, Google Scholar searches were run with combinations of these keywords: structural color, butterfly, Lepidoptera, iridescence, scale and Ghiradella. Database searches brought up tens of thousands of hits, many of which were not pertinent, so we used high-quality results to find cited and citing references and noted every species that was mentioned in connection with structural color, iridescence or derived scale morphology. Finally, a database search was run on each species name that had been mentioned in any prior included reference.

When a search on species name returned many results, it was searched again in combination with the keywords. If the search on species name returned no relevant results, we tried searches with only the genus name and checked for alternative nomenclature. We continued aggregating references and running database searches on species until we could no longer find any new taxa mentioned in connection with structural color. Criteria for inclusion were that the article must include either (1) reflectance measurements or (2) electron microscope images of non-transparent (i.e. colored) optical nanostructures in a butterfly. We also included studies that provided additional characterizations (e.g. absorption measurements, mathematical modeling, scatterometry; see Glossary) for structures that had been included on the basis of (1) or (2).

This literature review strategy yielded 187 included references which described 420 potential structures from 378 species (see Dataset 1 in Dryad, https://doi.org/10.5061/dryad.qnk98sfnx). From that initial list, we secondarily excluded entries that were presented as non-photonic comparisons with structurally colored specimens. Only seven reported structures occurred outside adult wing scales, which were all multilayer broadband reflectors (see Glossary) in the pupal cuticle (Neville, 1977; Steinbrecht, 1985; Steinbrecht et al., 1985). We therefore narrowed our focus to structures located in scales or bristles (Ghiradella, 1994; Tilley and Eliot, 2002) on the adult, which can be homologized and directly compared in subsequent analyses. After filtering, there were pairwise complete data on both color and morphology for 312 optical nanostructures from 286 species. Some species had multiple structural colors on different body parts (e.g. blue dorsal and green ventral wings in *Cyanophrys remus* and *Albulina metallica*; Biró et al., 2007).

To compare color between structures, we recorded the peak reflected wavelength (i.e. hue) and the percentage reflectance at that wavelength (i.e. brightness) for each structural color. Because of iridescence, quantification of structural color is extremely sensitive to the measurement protocol; specifically, illumination and detection angles, light source, reference sample and spot size (Meadows et al., 2011). Spectroscopy methodologies were variable among the included studies, making comparisons imperfect; nevertheless, the data are useful to show broad trends. When multiple spectra were available, we used the following rules for consistency. When reflectance was reported from more than one angle, the peak wavelength at the maximally reflective angle was used. If comparable reflectance data were reported from more than one study or from replicated specimens, we took their average. When reflectance data were found for both an isolated scale and the intact wing, both values were noted, but the intact wing reflectance was preferentially used in comparative analyses for consistency, because single scale reflectance measures were uncommon. In cases where structures produced two reflectance peaks – as in *Chrysozephyrus* species with both a UV and a green peak (Imafuku et al., 2002b) – the brighter peak was used in graphical summaries, but both were listed in the dataset (https://doi.org/10.5061/dryad.qnk98sfnx). Peak wavelengths were typically estimated by eye from graphs, which limited precision to a 5–10 nm window around the measured peak. This precision limit is similar to the magnitude of inter-individual variation (Imafuku et al., 2002a; Bálint et al., 2008). We also recorded percentage reflectance at the maximally reflective wavelength (i.e. spectral intensity or 'brightness'). When no reflectance spectra were available but a color image or a qualitative color descriptor (e.g. 'blue', 'UV') was given, the qualitative descriptor was recorded. Broadband reflectors have a similar reflectance intensity across many wavelengths, so the maximally reflecting wavelength is not a good summary of the reflector's properties and may not be identifiable from a visual inspection of a graph. Therefore, for broadband reflectors, we only recorded a qualitative descriptor, such as 'white', 'silver' or 'gold'. Additionally, some reddish lamina thin films that reflected in both violet and red, without a peak wavelength in either region, were handled as qualitatively 'magenta'. Note that many reflectance spectra were likely influenced by co-occurring pigments as well as the nanostructures (Box 1).

To compare scale morphological modifications, we noted which type of optical nanostructure (Fig. 1) was present. Generally, we followed the author's conclusion as to which scale component caused the optical properties. If the author's description was brief but a micrograph was provided, we assigned the structure to the same category as the well-studied examples that it most resembled. In a few cases when the proposed mechanism seemed questionable, we noted the explanation in the spreadsheet but dropped that structure from comparative analysis (for example, the proposed nanostructure was not present in the provided micrograph, or the mechanism was disputed across studies). Filled-in windows (Fig. 1F) and crossrib air columns (Fig. 1G) likely involve modifications to both the crossribs and microribs, and reflectance in these scales also requires the lower lamina; for simplicity, we have summarized them as crossrib structures.

Box 1. Relationship with pigments

Although structural and pigmentary colors have different underlying physical mechanisms, they frequently co-occur and interact. Among the surveyed literature, 86 descriptions of optical structures also reported a pigment to be present in the same scale, with an additional 11 structures in cover scales that interacted with a pigment in the underlying ground scale. We highlight four roles that pigments fill when interacting with a structural color.

(1) Color mixing: in *Colotis regina*, a blue multilayer and red pigment combine to make purple (Giraldo et al., 2008). Color mixing may also include UV structural colors, as in *Colotis danae* (Wijnen et al., 2007).

(2) Enhancing spectral purity: by absorbing off-color light that passes through the optical structure and could otherwise be subsequently backscattered, a layer of melanin under or behind the structure makes the reflected color more saturated (Giraldo et al., 2016; Siddique et al., 2016). Pterin pigments enhance spectral purity in *Colias eurytheme* (Rutowski et al., 2005).

(3) Spectral filtering: especially when positioned above the structure, pigments can selectively block reflectance to narrow the reflected wavelength range, eliminate circular dichroism (Wilts et al., 2012a) or suppress iridescence (e.g. papiliochrome II in *Papilio nireus*; Wilts et al., 2012b).

(4) Increasing the refractive index: chitin that contains melanin has a higher refractive index than pure chitin (Stavenga et al., 2012), which changes a reflective structure's behavior, as in structurally enhanced 'ultra-black' scales. 'Ultra-black' scales capture more light than can be explained by melanin's absorptive behavior alone, thanks to melanin's impact on the refractive index (Davis et al., 2020). Pterin pigments in Pierid granules raise the refractive index, which enables the granules' light scattering behavior and makes the granules simultaneously both a pigment and a structural color (Wilts et al., 2017b). Moreover, when only part of a chitin structure contains a chemical pigment, this can create additional reflective interfaces (Trzeciak et al., 2012; Prakash et al., 2023 preprint). All told, pigmentary and structural color are at times only separable in the abstract, and their integrated functions should be considered.

Nanostructure morphology

The literature review identified 318 optical nanostructures from 290 species whose location in the scale anatomy could be determined

(see Dataset 1 in Dryad, https://doi.org/10.5061/dryad.qnk98sfnx). Nearly all of these structures were documented by at least one micrograph, except where species in larger studies were simply described as having the same scale anatomy as a relative for which data were shown (Wilts et al., 2015; Giraldo et al., 2016). All but 10 structures belonged to the same category as one of the examples presented in Fig. 1, while allowing for variation in the number of layers in a multilayer, the angular orientation of the layers and the filling fraction of photonic crystals.

There were multiple examples of optical nanostructures in each scale part (Table 1). Perforated multilayers were the most common structure. Microrib structures were the least common, and the most likely to co-occur with another optical nanostructure elsewhere in the scale. Microrib structures were also morphologically heterogeneous. Most of the microrib structures were multilayers, reminiscent of the ridge multilayers; however, the angle of the multilayer relative to the scale lamina was more variable than among ridge multilayers. For example, in *Troides magellanus* and *Rapala manea*, microrib multilayers were oriented nearly vertically, which produced only grazing-angle reflectance (Lawrence et al., 2002; Tilley et al., 2002). *Trogonoptera brookiana* had a unique microrib multilayer with enlarged microribs that extended in parallel along the full scale (Wilts et al., 2016; Fig. 1E). Crossrib structures were also heterogeneous, including filled windows (similar to Fig. 1F) and vertical air columns (similar to Fig. 1G; all found in *Papilio* species). *Euptychia tricolor* and *Lamprolenis nitida* had flattened, tiled crossribs that formed a multilayer (Ingram and Parker, 2008). Among the continuous lumen multilayers, 6–10 in *Papilio* species had a tertiary structure of regular concavities, which create color mixing (Vukusic et al., 2000; Kolle et al., 2010; Fig. 1K). This last count is approximate because several micrographs looked potentially curved, without a conclusion on whether the curvature affected color.

A subset of the scales were more distinctive, having multiple optical structures, lost scale parts, or other peculiarities. Two species' scales had lost the microribs and crossribs (*Archon apollinus* and *Parnassius hardwickei* (Ingram and Parker, 2008), and were reported to have violet reflectance (optical mechanism is unknown). More severely reduced scales had only a single lamina remaining (*Elbella polyzona*; Ghiradella, 2010). In certain scales, the anatomy of each part was typical, but the scale's orientation changed the optical effects. *Carystoides escalantei*'s scales had typical anatomy but stood vertically, perpendicular to the wing membrane, with both the microribs and crossribs suggested to cause incoherent scattering (Ge et al., 2017). In *Pierella luna*, the apical tip of each scale curled over, making the crossribs form a vertical grating (Vigneron et al., 2010; England et al., 2014). A few scales contained multiple photonic structures, which can reinforce or add complexity to the optical signal. For example, *Chliaria othona* had both a perforated multilayer and a microrib multilayer (Tilley et al., 2002). *Lamprolenis nitida*, *Caligo memnon* and *Caligo beltrao* had two multilayer gratings that slanted in opposing directions, potentially creating two separate optical signals (depending on the illumination and viewing contexts). In *L. nitida*, the double gratings are microrib and crossrib multilayers (Ingram et al., 2008), while scales in the *Caligo* species contain both ridge and microrib multilayers (Ghiradella, 1991, 1994). All scales include a lower lamina, but to date, only two studies have specified that thin film effects from the lower lamina contribute to total reflectance in scales that also contain another optical structure (*Heliconius sara*: Wilts et al., 2017c; and *Morpho deidamia*: Giraldo et al., 2016; these are plotted as ridge structures in Figs 2–4). Because the lower lamina is a conserved scale element, lamina thin films likely influence reflectance alongside other optical structures more often than has been reported. Many of these peculiar scales have only been described with a single micrograph and are excellent candidates for further investigation.

Color range

To define the color gamut that butterfly optical nanostructures produce, we recorded the hue of each specimen, using the quantitatively measured wavelength of maximal reflection when possible (Fig. 2, circles) and a qualitative descriptor of color (Fig. 2, triangles) when no spectra were available and for broadband reflectors (e.g. white, silver, pearlescent; see Dataset 1 in Dryad, https://doi.org/10.5061/dryad.qnk98sfnx). Broadband reflectance and colors with peak wavelengths between 350 and 550 nm (i.e. UV, blue, green) were common. By contrast, long-wavelength structural colors were extremely rare. Only three structures had a defined spectral peak with maximum reflectance at or above

Table 1. Counts of optical nanostructures by category

Location/type	Count	Representative example	Corresponding panel of Fig. 1
Ridge multilayer	68[a]	*Morpho cypris*	D
Microrib	16	*Troides magellanus*	E
Crossrib			
Bilayer with lower lamina[b]	27	*Argyrophorus argenteus*	F
Bilayer+air columns	4	*Papilio nireus*	G
Multilayer	1[c]	*Euptychia tricolor*	
Lumen			
Continuous multilayer	46	*Ornithoptera croesus*	K
Perforated multilayer	78	*Polyommatus icarus*	I
3D photonic crystal	23	*Callophrys rubi*	J
Lower lamina thin film	43	*Junonia coenia*	B
Other			
Scattering pigment granules	3	*Pieris rapae*	H
Reduced scales	3	*Elbella polyzona*, *Archon apollinus*	
Vertical/curled	2	*Carystoides escalantei*, *Pierella luna*	
Combination	4	*Lamprolenis nitida*, *Chliaria othona*	

[a]Count includes two scales where a lamina thin film also influences the reflectance. [b]All are broadband reflectors. Although the solid upper surface may be derived from both microribs and crossribs in some cases, we summarize these as crossrib structures for simplicity. [c]See also *Lamprolenis nitida* in the 'other: combination' category.

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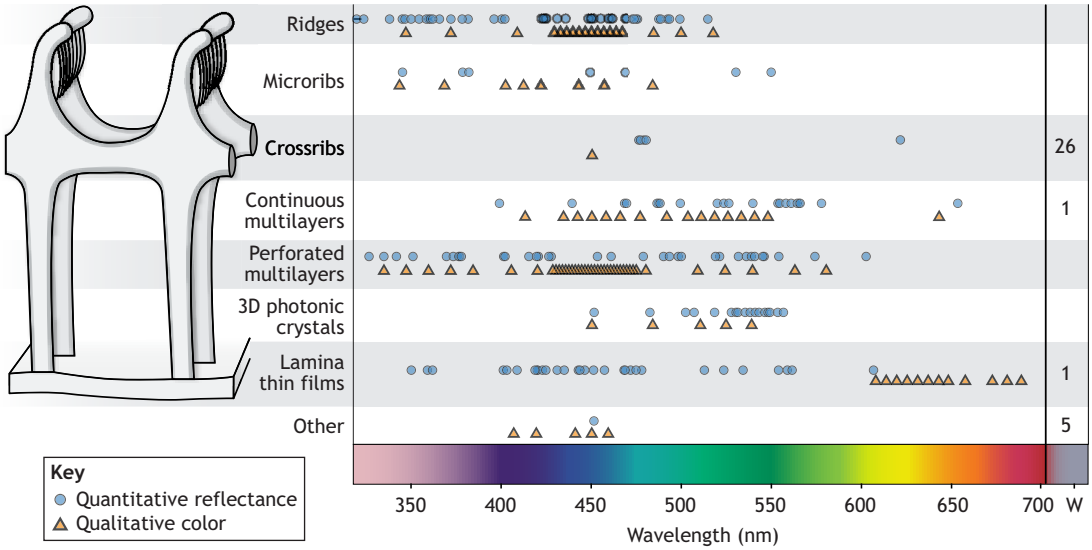

Fig. 2. The color range of butterfly optical nanostructures in each morphological category. Circles represent quantitatively measured wavelengths of maximal reflection for one photonic structure. Triangles represent qualitative color descriptions for structures whose reflectance spectra were unavailable, or which produced broad reflectance without a defined maximal wavelength (e.g. copper, gold). Counts of white, silver and pearlescent structures are shown in the 'W' column. Qualitative data points (triangles) are evenly distributed within these ranges: 'UV' is 320–390 nm; 'violet' is 390–430 nm; 'blue' is 430–470 nm; 'blue–green' is 470–500 nm; 'green' is 500–550 nm; 'yellow–green' is 550–590 nm; 'yellow' or 'gold' are 590–620 nm; 'orange', 'bronze' or 'copper' are 620–665 nm; and 'red', 'pink' or 'magenta' are 665–700 nm. Illustration credit: Dennis Sun. Note that co-occurring pigments may also influence color measurements (Box 1).

600 nm. Of these, the longest wavelength structure belonged to *Ornithoptera croesus lydius*, with maximal reflectance at 650 nm (Zhang et al., 2014; Kazama et al., 2017). The structural color with the next longest wavelength was the golden crossrib and lamina bilayer of *Anteros formosus* at 621 nm (Ren et al., 2020), followed by a perforated multilayer in *Jalmenus evagoras* with peak reflectance around 610 nm (Wilts et al., 2009). However, *J. evagoras* had a relatively broad reflectance peak, and by eye looked a weakly saturated green color. Additionally, a continuous lumen multilayer structure was qualitatively described as 'bronze'

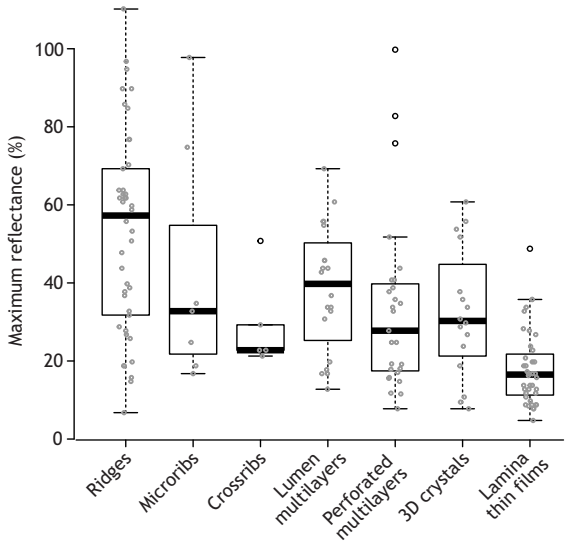

Fig. 3. The percentage reflectance at the maximally reflective angle and wavelength for different types of optical nanostructures. Boxplots show median and inner quartile range, whiskers extend to 1.5 times the interquartile range, and outliers are shown as circles. Broadband reflectors are not included (see 'Meta-analysis methods').

(*Heliophorus brahma*; Ingram and Parker, 2008), and eight thin films were broadly reflective in long wavelengths, resulting in golden and copper colors (Stavenga et al., 2014a,b; Thayer et al., 2020). There were several reports of structurally enhanced black (Vukusic et al., 2004; Sackey et al., 2018), in which internal reflection allows scales to capture more light than can be explained by melanin's absorption alone (~0.1% of light reflected in 'ultra-black' versus ~1% reflected in the comparison set; Davis et al., 2020). All 'ultra-black' scales were melanic with morphologies that occur in non-black scales as well.

The color red deserves special consideration. It is striking that although red is a common wing color, only four structural colors can be described as reddish. The best example – *Ornithoptera croesus lydius* – had lumen multilayers with reflectance varying among individuals from orange to red (Zhang et al., 2014; Kazama et al., 2017). However, reflectance in *O. croesus* scales also requires a filtering pigment that absorbs blue light (Wilts et al., 2015). Less compelling examples include two lamina thin films with modest reflectance in both the far red and violet wavelengths that combine to a dim magenta (Thayer et al., 2020). *Lamprolenis nitida* males reflect brightly at 700–800 nm, but only under artificial lighting conditions: the wing must be both illuminated and viewed from a grazing angle in the anteroposterior direction (Ingram et al., 2008). Yet, better examples of structural red can be found by extending the search to non-butterfly Lepidoptera. For instance, sunset moths have red scales with multilayers that reflect at 700–800 nm (Yoshioka and Kinoshita, 2007), and, under high humidity, the green forester moth's multilayer structure reflectance approaches 700 nm (Wilts et al., 2019). Taken together, structural red is developmentally possible in Lepidopteran scales, but red pigments predominate.

Intersection of morphology and color

We next compared color across the optical nanostructure categories (Fig. 2). The most accessible section of the color spectrum was

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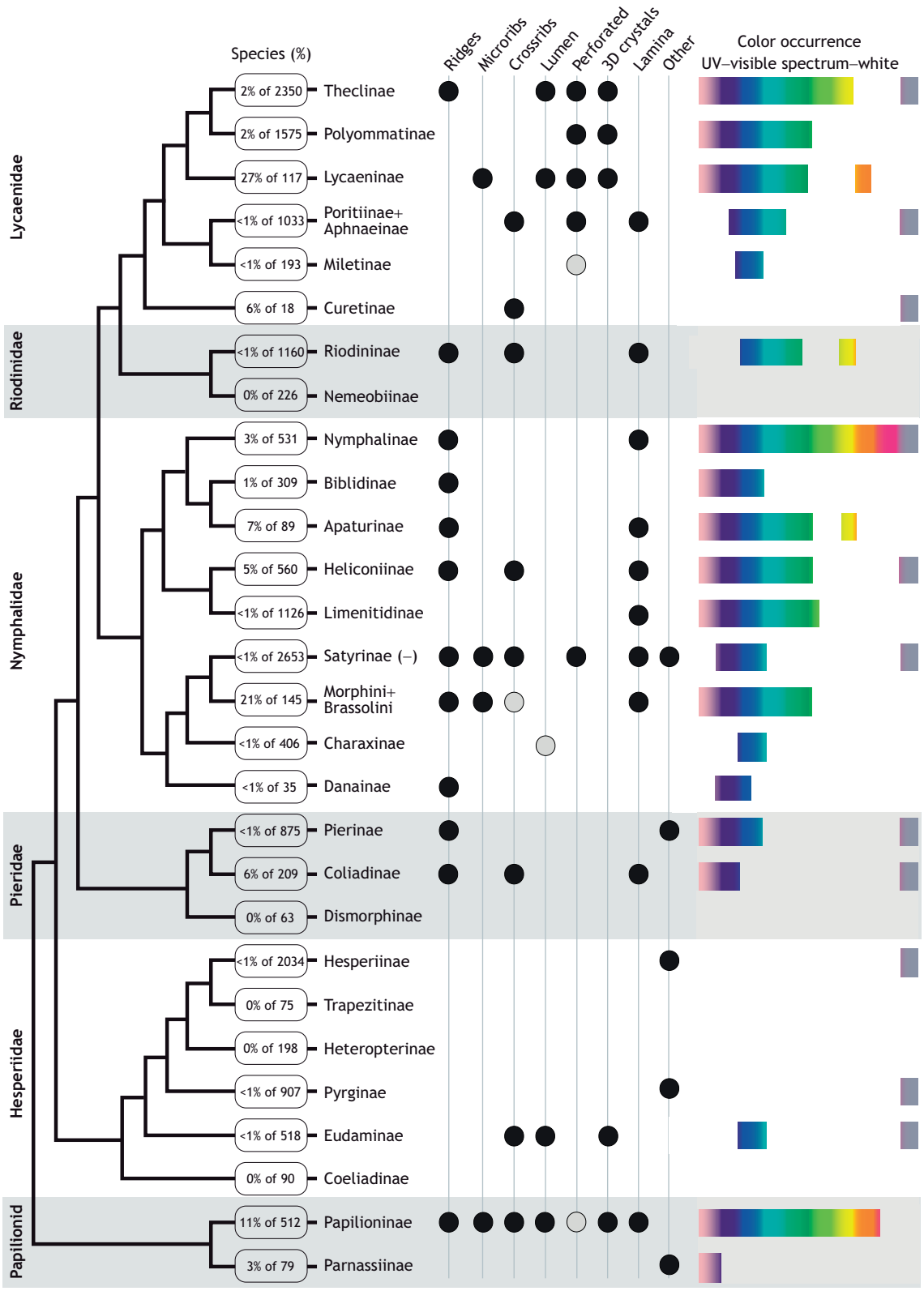

Fig. 4. See next page for legend.

450–500 nm (i.e. blue), as these wavelengths were generated by structures in every part of the scale. Lamina thin films produced the broadest color range, making them the most versatile structures in terms of wavelength. Only the ridge, microrib, lamina and perforated multilayer categories included structures with maximal reflectance in the UV. No ridge, microrib or 3D photonic crystal structures reflected long-wavelength colors. Crossribs and 3D photonic crystals had the most restricted color ranges: crossrib structures primarily produced broadband reflectance, while 3D photonic crystals were restricted to 440–560 nm. These defined

Fig. 4. The phylogenetic distribution of structural colors. Left: a cladogram (based on Espeland et al., 2018), including subfamilies with at least one characterized structural color or more than 50 species. The Morphini+Brassolini clade is displayed separately from the rest of Satyrinae because it has been intensively studied. Bubbles on cladogram branches contain the approximate number of species per subfamily and the percentage of those species for which a structural color has been characterized, rounded to the nearest integer. Species count estimates are based on Callaghan et al. (2004), Markku Savela's website (www.funet.fi/pub/sci/bio/life/insecta/lepidoptera/ditrysia/papilionoidea/) and Niklas Wahlberg's nymphalidae.net database, which compiles findings from dozens of studies (e.g. Peña et al., 2006; Heikkilä et al., 2012). Black circles in the middle section show the types of structures that occur in each subfamily. A gray circle means there is suggestive evidence that the structure occurs (e.g. the structure was mentioned in a larger work without data; the specimen's identity was imprecise). Right: the structural color gamut for each subfamily is shown by a color bar that reads (from left to right): UV, the visible spectrum from violet to red, and lastly broadband reflection (i.e. white, silver).

color ranges can be used to gauge the novelty of newly characterized structures.

We also examined the relationship between brightness (i.e. percentage reflectance at the maximally reflective wavelength) and morphology (Fig. 3). Because spectroscopy protocols are highly variable among studies, and because the sample size was small for some morphological classes, we graphically summarized the literature but did not apply a statistical model across classes. We observed suggestive differences among the structural classes that raise testable hypotheses for future work. Most dramatically, lamina structures are the least bright, which is consistent with mathematical models for simple thin film reflectors. This brightness performance limitation contrasts with lamina thin films' color range versatility and helps explain what elaborate structures offer beyond simple thin films. There also seem to be differences in brightness variability between categories. In particular, categories with multilayers (ridge, microrib and lumen multilayers) have higher mean reflectance, but also the most variability, which might be explained by variation in the number of layers per multilayer. Reflectance increases with the number of overlapping layers per multilayer (Kinoshita and Yoshioka, 2005), consistent with both the large variation within multilayer categories and the low reflectance from lamina thin films, which are monolayer structures. Another factor known to modulate brightness in ridge multilayer structures is ridge density (Parnell et al., 2018). Rigorously testing what causes brightness variation by re-measuring diverse structures with a uniform spectroscopy protocol and considering the number of overlapping layers, the influence of any co-occurring pigments (see Box 1) and the structure's position relative to the top of the scale would be interesting. Combining data from all morphological categories, we found no association between percentage reflectance and wavelength (Pearson's correlation, r=−0.13, P=0.11), suggesting that differences in reflection efficiency do not explain why short wavelengths predominate.

Phylogenetic distribution of structural color

To investigate whether optical nanostructures are overrepresented in certain lineages, we compared the allocation of structural colors among butterfly subfamilies (Fig. 4). We found that optical nanostructures are unevenly distributed among subfamilies, especially when considering subfamily size. For example, Lycaeninae and Morphini (a clade within the subfamily Satyrinae) have relatively few species, but have the highest frequencies of structural coloration. In contrast, the large Hesperiinae, Pyriginae and the non-*Morpho* Satyrinae subfamilies have few characterized structural colors. Possibly, clades with the highest-reported frequencies of structural colors actually have a higher incidence of structural coloration. More likely, the current distribution highlights taxa that remain understudied.

Scale anatomy has been investigated in very few of the ∼18,000 butterfly species, each of which bears multiple scale types. Additionally, there are uncharacterized blue, green and iridescent wing displays among the less-studied clades, such as Hesperids (Evans, 1949; Li et al., 2019). An attention bias in favor of groups such as Morphos, Lycaeninae and Papilionids might be explained by the early discoveries of elaborate structures of species in these clades, generating sustained interest in their relatives. Yet now, more research on structural color in understudied groups is needed to determine the evolutionary origins and patterns of trait convergence in photonic nanostructures. Less-studied groups, including small subfamilies, may present the best opportunities to find novel structures, because their independent evolutionary histories allow for morphological divergence.

Although further work on the phylogenetic distribution of structural color is needed, overlaying morphology and color on the phylogeny revealed some interesting patterns (Fig. 4). Blue wavelengths occurred in more subfamilies than did the rest of the spectrum, although all hues from UV to green were common, especially among the subfamilies with more than a few described structures. This phylogenetic blue dominance complements the finding that blue was produced by more morphological categories than other wavelengths (Fig. 2). Nymphalinae was the only lineage whose structures spanned the full color range, despite having only two kinds of structures. Papilioninae included the most morphological diversity, with optical structures in every part of the scale. Satyrinae also showed high morphological diversity, despite having only 13 characterized optical structures. Ridge multilayers, lamina thin films and broadband-reflecting crossrib bilayers (similar to Fig. 1F) occurred in the greatest number of subfamilies. For lamina thin films, this broad occurrence most likely reflects conservation of the ancestral morphology (Thayer et al., 2020), while modified crossribs are thought to be convergently evolved (Ren et al., 2020). By contrast, perforated multilayers were taxonomically constrained, occurring almost exclusively in Lycaenidae, even though they were the most commonly reported structure (one exception in Satyrinae: *Euptychia cephus*: Ingram and Parker, 2008; and a second possible example with slight perforations in Papilionidae: *Parides aeneus*: Wilts et al., 2014). Strikingly, most of the peculiar scales ('other' in Fig. 4 and described in the 'Nanostructure morphology' section) were found in subfamilies that have been infrequently studied, further indicating that such taxa deserve more attention. Denser, phylogenetically strategic sampling is needed to determine the timing and number of independent origins for each type of nanostructure.

Forces that shape the color gamut

A striking observation of this meta-analysis is that structural color is ubiquitous in short wavelengths, but extremely rare in long wavelengths – especially red. Blue (around 450 nm) occurs in more butterfly lineages and is produced by more kinds of nanostructures than other hues. This skewed color range may imply functional tradeoffs that limit either the production or the relative utility of long-wavelength photonic structures. Butterfly lamina thin films cannot generate a bright or spectrally pure red profile (Thayer et al., 2020), and physical constraints prevent a specific kind of structural red in other animal taxa (Magkiriadou et al., 2014). Exploring whether constraints also exist at long wavelengths for other butterfly nanostructure categories is an

exciting prospect. For example, future work could test whether structures at the extremities of each category's color range have poorer mechanical (Large et al., 2009), thermal (Krishna et al., 2020), hydrophobic (Wagner et al., 1996; Zheng et al., 2007) or self-cleaning (Köchling et al., 2020) properties. These measurements could be integrated with modeling work, which could identify the dimensions required to produce long-wavelength structures not observed in nature (e.g. the dimensions of a ridge multilayer that reflects in yellow) and infer their biomechanical properties. Such comparisons might uncover specific functional tradeoffs. Alternatively, these models may find that the missing long-wavelength photonic structures are optically outperformed by long-wavelength pigments. Ultimately, genetic manipulations or drug treatments could be used (similar to Null, 2017; Lloyd et al., 2023 preprint) to provoke exaggerated structures outside the naturally occurring range and observe their properties, including whether scale development can proceed to maturity.

A reciprocal hypothesis is that the pigment color gamut is primarily constrained in short wavelengths, and the color range observed among photonic structures is compensatory. This framework is similar to the avian plumage color gamut, in which structural colors access a much larger proportion of color space (see Glossary) than do avian pigments (Stoddard and Prum, 2011). While blue is the dominant structural color among butterflies, blue pigments are rare in adult butterfly wings, and somewhat rare among animals generally (Simonis and Berthier, 2012), possibly owing to challenging biosynthesis. The limitations on short-wavelength pigments could be probed with an analysis of larval colors. Cryptic green is a common larval color (Medina et al., 2020) and blue also occurs, sometimes in iridescent larval ornaments. While few lepidopteran larval colors have been characterized as pigmentary or structural, reports exist of both blue–green larval bilin pigments (Wieland and Tartter, 1940; Barbier, 1981) and Tyndall scattering in tent caterpillars (Byers, 1975). If larval bilin pigments are widespread across taxa, then biosynthesis is not the key limitation. Indeed, if blue–green larval pigments are equally prevalent among species whose adults bear blue–green wing structures, that would point to differences in the feasibility of embedding pigments in scales versus forming nanostructures in integument. The prevalence and attributes of integumentary nanostructures in butterfly larvae are unknown, but the case can be made that embedding blue pigments into scales is challenging. Poor solubility properties have complicated efforts to extract and identify blue pigments (Simonis and Berthier, 2012), and may similarly complicate deploying them within scales. Of the few species with blue, pigment-based wing displays, most (e.g. *Tirumala hamata*, *Papilio phorcas* and *Graphium* species) have the bilin pigment localized in the wing membrane rather than the scales (Barbier, 1983; Rothschild and Mummery, 1985). These pigmented membranes are accompanied by adaptations that expose the membrane, including scale transparency or reduction to bristles (Rothschild and Mummery, 1985; Stavenga et al., 2010). Thus, even the exceptional blue pigmentary wing displays may emphasize the rule: it is often easier to remove or reduce scales, exposing a pigmented membrane, than to pigment the scales themselves. Note, however, the exceptional *Nessaea* butterflies, which do have blue pigmented scales (Vane-Wright, 1979; R.C.T., personal observation).

Biological processes that generate structural color

Evolution

This Review provides an attempt to look holistically at the phylogenetic distribution of structural color across butterflies, but variation among smaller sets of closely related species has been investigated in the following genera and tribes: *Argynnini* (Simonsen, 2007), *Cyanophrys* (Bálint et al., 2009), *Heliconius* (Wilts et al., 2017c; Parnell et al., 2018), *Junonia* (Thayer et al., 2020), *Morpho* (Giraldo et al., 2016; Null, 2017), *Ornithoptera* (Kazama et al., 2017), *Papilio* (Null, 2017), *Parides* (Wilts et al., 2014) and *Polyommatus* (Bálint et al., 2012). Overall, genera-focused studies find that similar structures exist in many related species, suggesting that the structures either originated before the surveyed genera diversified or early within the clade. Smaller modifications to the nanostructure (e.g. thickness, number of layers) are common within these genera. In addition to providing evolutionary insight, these datasets are among the best resources for unravelling how each dimension of a structure affects its optical properties. *Parides* and *Argynnini* show complex patterns of presence/absence of the focal structures within the study set, but a straightforward interpretation of the number of gains and losses was not possible in either case. The idea that photonic structures may easily switch in and out of use across a phylogeny is also supported by CRISPR mutagenesis studies that find wholesale gain of a complex structure upon knocking out a single repressor gene. Indeed, *bric-a-brac* deficiency revealed an atavistic gain of dense, many-layered, well-formed ridge multilayers in *Colias philodice*, a species that does not naturally display a ridge multilayer (Ficarrotta et al., 2022). This result means that the genetic program to build a structure can be conserved in the structure's apparent absence.

Variation within and between populations is the substrate on which evolutionary selection acts, but it has rarely been examined. To date, most butterfly structures have been characterized from a single individual, and often from only one scale. More replication is not only desirable for robust conclusions but also a key opportunity to advance evolutionary research. The most thorough series of work on variation in Lepidopteran photonic structures carefully outlines population-level and plastic variation (see Glossary) in *Polyommatus* butterflies. The spectra of *P. icarus* butterflies are strikingly stable across seasons for over 100 years within Hungary, but vary biogeographically across Eurasia (Kertész et al., 2019). In *P. dorylas*, photonic structures do not vary between bivoltine lowland and univoltine upland populations (Bálint et al., 2019). Although *P. icarus* has cold stress-induced plastic responses (e.g. melanization), photonic structure reflectance is not plastic in males (Kertész et al., 2017), but induces ectopic blue scales in females. *Zerene* butterflies also showed limited structural color plasticity: larval diet had a small effect on the density of scales bearing photonic structures, but did not affect the dimensions of the ridge structure (Fenner et al., 2019). Nanostructure variation among geographical races and subspecies has been described in *Heliconius* (Brien et al., 2019; Curran et al., 2020) and *Euphaedra* butterflies (Bálint et al., 2023). Lastly, *Bicyclus anynana* (Wasik et al., 2014) and *Junonia coenia* (Thayer et al., 2020) show rapid evolutionary responses to artificial selection on structural color, increasing the thickness of thin film laminae, which produces violet and blue reflectance. These rapid evolved responses point to selectable standing variation in the studied populations before artificial selection, a useful contrast to the largely invariant structures within *Polyommatus* populations. More studies on how structures vary between individuals and populations will be crucial because variation at this level is the most useful tool to uncover genetic control of the structures (i.e. using association mapping, breeding experiments or comparative transcriptomics). Additionally, patterns of variation can suggest the ecological function of a structure. For

example, sexually dimorphic structural colors could play a role in courtship, while geographic variation might indicate a role in local environmental adaptation.

Development

Relatively little is known about how photonic structures in butterfly scales develop, but key events in the development of standard scales have been described, providing a helpful baseline. Scale development begins with the specification of scale cells by Delta-Notch signaling and *As-c* expression, homologous to the specification of sensory bristles in *Drosophila* (Galant et al., 1998; Reed, 2004). As development proceeds, the scale cell grows apically outward from the wing membrane (Stossberg, 1938), and actin rods, which are regularly arranged intercalating the future positions of ridges, appear to assist with apical extension (Dinwiddie et al., 2014; Day et al., 2019). Once the scale cell has lengthened, it flattens, which may be mediated by microtubules (Overton, 1966; Greenstein, 1972). Finer structural elaboration occurs, then chitin is deposited. In papilionid scales that have reticulated crossribs at maturity, actin bundles are reorganized after ridge development to resemble the crossrib reticulation, possibly mediated by Arp2/3 (Seah and Saranathan, 2023). The actin bundles subsequently break down, and pigments are deposited shortly before the adult's emergence from the pupa (Koch et al., 1998; Iwata et al., 2014). Recent advances in live imaging help to define the timing of these events (Iwata et al., 2014; Null, 2017; McDougal et al., 2021).

Time-series images of developing scales exist for three kinds of photonic scales: gyroids, lumen multilayers and ridge multilayers. Ghiradella (1989) took transmission electron microscopy (TEM) micrographs of developing scales containing gyroids (*Callophrys rubi*). These gyroid scales have expansive and convoluted endoplasmic reticulum membranes, which appear to provide an organizing template for the crystal domains. Saranathan et al., (2010) added small angle X-ray scattering data alongside the micrographs to develop a model wherein scale cells first form a thermodynamically favorable double gyroid out of lipid membranes and cytoplasm, which is subsequently converted to a more efficient single gyroid via chitin deposition. Moreover, because discrete domains of crystals are biggest at the apical tip of mature scales, it is believed that apical crystallites begin developing earlier (Ghiradella, 1989; Wilts, et al., 2017a). Building on the earlier finding that actin rods intercalate nascent scale ridges, Null (2017) showed that hexagonal arrays of actin cables also prefigure the edges of dimples in the dimpled multilayers of *Papilio palinurus*. Whether ridge multilayers are also pre-patterned by either membranes or the cytoskeleton is still being explored. Actin-inhibiting drug treatments in developing *Heliconius sara* scales show that the cytoskeleton plays a major role in ridge multilayer development (Lloyd et al., 2023 preprint). A micrograph time series of developing ridge multilayers in *Colias eurytheme* (Ghiradella, 1974) prompted the alternative hypothesis that ridges form by mechanical buckling under tension, which could involve the cytoskeleton in lengthening the scale to generate tension, rather than in directly providing a template for chitin deposition.

Genetic regulation

Relatedly, the molecular and genetic regulation of optical nanostructures is largely mysterious, but recent advances indicate that the nanostructures are jointly regulated by high-level patterning genes that also control pigmentation. The earliest work on the genetics of butterfly structural colors tracked inheritance patterns in experimentally crossbred *Colias* butterflies and concluded that the presence of UV-reflective ridge multilayers in males was controlled by a single sex-linked gene (Gerould, 1943; Silberglied and Taylor, 1973), which was recently identified as *bric-a-brac* (Ficarrotta et al., 2022). A pair of studies used CRISPR/Cas9 mutagenesis in *Junonia* butterflies to show that *optix* determined whether scales produced thick, blue lamina thin films or thinner golden ones (Zhang et al., 2017; Thayer et al., 2020). A third study in *Bicyclus* found five genes (*Antennapedia*, *apterousA*, *doublesex*, *Ultrabithorax* and *optix*) for which deficiency mutants either gained or lost broadband silver reflectance via a continuous upper scale surface (i.e. filled windows, similar to Fig. 1F), often with corresponding effects on lower lamina thickness (Prakash et al., 2022). Correlations between gene expression and nanostructure presence suggest that *doublesex* controls ridge multilayer specification in *Zerene*, although a causal relationship could not be confirmed via genetic engineering because gene deletion was lethal (Rodriguez-Caro et al., 2021).

These implicated genes share many commonalities: all are transcription factors, regulating the expression of downstream genes (see Glossary), and all are well known for their roles in arthropod morphogenesis [e.g. body organization and segment identity (Averof and Patel, 1997), dorsal identity in the wing disc (Cohen et al., 1992; Prakash and Monteiro, 2018), sex comb and ovary development (Couderc et al., 2002), eye formation (Seimiya and Gehring, 2000)]. In butterflies, the deficiency mutants showed discrete, large-effect switches between two different identities. For example, the results describe the presence or absence of fully formed ridge multilayers, rather than subtler effects on multilayer height and organization; a discontinuous jump between thin gold and thick blue laminae, with no observations of intermediate, reddish thicknesses; and switches between fully solid and ∼40% open upper surface area, corresponding to the presence or absence of silver reflectance. Moreover, these scale identity switches included suites of phenotypes beyond the morphology of the nanostructure, such as changes to pigment abundance, pigment identity, or total scale size or scale shape (i.e. scalloped or tapered tip). Also, for most of the genes (*optix*, *dsx*, *bab*, *apA*), deficiency phenotypes depended on context, with different effects – even opposite direction effects – depending upon the wing region (e.g. dorsal or ventral surface, forewing or hindwing, within an eyespot). Together, these findings paint a picture of high-level regulatory genes that control wing patterning by determining scale identity in a region-specific manner, where structural color is one of many jointly coordinated aspects of scale identity. This behavior conveys a complex regulatory architecture, in which the focal genes integrate multiple upstream inputs (i.e. information about wing region) and regulate a set of downstream activities relating to nanostructures, pigments, and scale size and shape. Coordinated patterning is sensible, as appropriate optical signals often require specific combinations of pigments and nanostructures (Giraldo et al., 2008; Wilts et al., 2012b; Thayer et al., 2020). This integrative regulatory architecture may allow adaptive evolutionary change to toggle between favorable groupings of scale attributes, while bypassing maladaptive combinations.

While familiar patterning genes determine which scale cells build optical nanostructures, the genetic program that actually builds the structure is still required. The latter is of broad interest across several disciplines, as it may facilitate biomimetic engineering innovations (Zhang et al., 2015; Wu et al., 2016). Currently, we lack the technology to synthesize photonic structures with the complexity and efficiency achieved by animals. Identifying the structure-building genes and their roles may help answer long-standing

questions about which synthetic assembly methods most resemble natural assembly, such as whether assembly is assisted by a template or the interface of two different fluids (Qi and Zhang, 2022), and whether it involves additive deposition or subtractive sculpting.

We can pose several expectations about the genes directly involved in building the nanostructures. First, they should be downstream of the wing patterning transcription factors in the genetic pathway. Additionally, allelic variation and deficiencies in these genes are likely on average to have smaller phenotypic effect sizes that are more specific to nanostructure morphology, although this is not a firm rule. No such genes have been positively identified; however, Brien et al. (2022) used linkage mapping and comparative transcriptomics between pairs of color variants with and without ridge multilayers in *Heliconius erato* and *Heliconius melpomene* to identify some candidate genes. Candidate genes were differentially expressed between color patches or located within quantitative trait locus confidence intervals, and included genes encoding enzymes (*O-GlcNAc transferase*, *Transglutaminase*, *Fatty acid synthase*) and genes with roles in cuticle dynamics (*chitin deacetylase 1*, *miniature*, a cuticle-protein-like gene) and cytoskeletal activity (*dynein heavy chain 6*, *ringmaker*, *trio* and an F-actin-uncapping protein). It is too soon to say whether these candidates are more compatible with a specific mode of synthesis, and some classes of genes could plausibly contribute via multiple mechanisms. For example, the cytoskeleton could help form a template for chitin deposition, or it could stretch the cell along one axis, resulting in tension and mechanical buckling. Another study in *Bicyclus* knocked out eight enzymes in pigment biosynthesis pathways and found that three genes (*yellow*, *ebony* and *DDC*) had effects on the spacing of ridges or crossribs (Matsuoka and Monteiro, 2018). Neither the wild-type nor mutant scales in this study were photonic, but a similar effect in scales with a ridge multilayer could alter its brightness, indicating that these genes could potentially influence photonic structure development in certain contexts. RNAi-mediated knockdown of four cuticle proteins and laccase2 in *Bombyx mori* also disrupted non-photonic scales, resulting in abnormal junctions between ridges and crossribs (Liu et al., 2021). Further progress can be made with additional forward and reverse genetic screens, as well as by using techniques such as ChIP-Seq to identify downstream targets of the wing patterning genes.

Conclusions and future directions

Major findings during the last century of research on butterfly structural colors include the identification of recurring morphological categories of nanostructures and their optical mechanisms. Additionally, we have an emerging picture of macroevolutionary patterns, including the phylogenetic distribution and color range for each kind of optical nanostructure. Time series and live imaging advances have broken ground on describing the developmental processes that generate photonic nanostructures, while geneticists have found that the allocation of photonic nanostructures across a wing is jointly regulated by wing patterning genes that also regulate pigmentation and other scale attributes.

Exciting prospects await for the next century of research on butterfly structural colors. Additional strategic sampling of natural variation in the structures, both within and among species, will support advances across disciplines. Variation among understudied taxa, particularly Hesperid butterflies, subadult life stages and moth outgroups, can clarify macroevolutionary questions such as the number and timing of independent origins of each type of structure. Documenting patterns of variation can support connections with the ecological functions of the structures and selective forces that influence them, which can be followed with experimental testing in the context of animal visual systems. Meanwhile, uncovering within-species variation can reveal taxa that are well suited for developmental and genetic studies. Major questions remain about how scale development is modified to produce diverse photonic structures. New opportunities should move beyond describing nanostructures toward experimentally manipulating them. One top priority is to understand the biological production of photonic nanostructures, including the identities and roles of genes involved in directly building the structures, with relevance for biomimetic engineering. Ultimately, genetic inroads will unlock a new array of experimental tools to explore the functional and developmental limits of the structures.

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Competing interests

The authors declare no competing or financial interests.

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Data availability

Data, analytical code and metadata are available from Dryad (Thayer, 2023): https://doi.org/10.5061/dryad.qnk98sfnx.

ECR Spotlight

This article has an associated ECR Spotlight interview with Rachel Thayer.

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