BioOne COMPLETE

Color Changes Upon Cooling of Lepidoptera Scales Containing Photonic Nanoarchitectures, and a Method for Identifying the Changes

Authors: Tamáska, István, Kértész, Krisztién, Vértesy, Zofia, Bálint, Zsolt, Kun, András, et al.

Source: Journal of Insect Science, 13(87) : 1-15

Published By: Entomological Society of America

URL: https://doi.org/10.1673/031.013.8701

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://complete.bioone.org/journals/Journal-of-Insect-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

Journal of Insect Science
www.insectscience.org

Color changes upon cooling of Lepidoptera scales containing photonic nanoarchitectures, and a method for identifying the changes

István Tamáska[1a], Krisztián Kertész[1b], Zofia Vértesy[1c], Zsolt Bálint[2d], András Kun[2e], Shen-Horn Yen[3f], László Péter Biró[1g*]

[1]Institute for Technical Physics and Materials Science, Research Centre for Natural Sciences, P.O.B. 49, H-1525 Budapest, Hungary
[2]Hungarian Natural History Museum, Baross utca 13, H-1088 Budapest, Hungary
[3]Laboratory of Natural Resource Conservation, Department of Biology and Institute of Life Science, National Sun Yat-Sen University, Kaohsiung, Taiwan, R. O. C.

Abstract

The effects produced by the condensation of water vapor from the environment in the various intricate nanoarchitectures occurring in the wing scales of several Lepidoptera species were investigated by controlled cooling (from 23° C, room temperature to -5 to -10° C) combined with *in situ* measurements of changes in the reflectance spectra. It was determined that all photonic nanoarchitectures giving a reflectance maximum in the visible range and having an open nanostructure exhibited alteration of the position of the reflectance maximum associated with the photonic nanoarchitectures. The photonic nanoarchitectures with a closed structure exhibited little to no alteration in color. Similarly, control specimens colored by pigments did not exhibit a color change under the same conditions. Hence, this method can be used to identify species with open photonic nanoarchitectures in their scales. For certain species, an almost complete disappearance of the reflectance maximum was found. All specimens recovered their original colors following warming and drying. Cooling experiments using thin copper wires demonstrated that color alterations could be limited to a width of a millimeter or less. Dried museum specimens did not exhibit color changes when cooled in the absence of a heat sink due to the low heat capacity of the wings.

Keywords: condensation
Correspondence: [a] tamaska.istvan@ttk.mta.hu, [b] kertesz.krisztian@ttk.mta.hu, [c] vertesy.zofia@ttk.mta.hu, [d] balint@zoo.zoo.nhmus.hu, [e] kuni@nhmus.hu, [f] shenhornyen@hotmail.com, [g] biro.laszlo@ttk.mta.hu, *Corresponding author
Editor: Jurgen Ziesmann was editor of this paper.
Received: 2 February 2012 **Accepted:** 29 September 2012
Copyright: This is an open access paper. We use the Creative Commons Attribution 3.0 license that permits unrestricted use, provided that the paper is properly attributed.
ISSN: 1536-2442 | Vol. 13, Number 87

Cite this paper as:
Tamáska I, Kertész K, Vértesy Z, Bálint Z, Kun A, Yen S-H, Biró LP. 2013. Color changes upon cooling of Lepidoptera scales containing photonic nanoarchitectures, and a method for identifying the changes. *Journal of Insect Science* 13:87. Available online: http://www.insectscience.org/13.87

Downloaded From: https://complete.bioone.org/journals/Journal-of-Insect-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

Introduction

Animal color is mostly due to the spectrally selective reflection of incident light. It can be associated with absorbing pigments (Nijhout 1991; Zollinger et al. 2003), which are often referred to as chemical color, and/or selective reflection caused by structural properties, which is usually referred to as physical or structural color (Joannopoulos et al. 2008; Biró and Vigneron 2011). Some physical colors can be produced by photonic crystals (Ghiradella and Butler 2009; Seago et al. 2009; Shawkey et al. 2009a; Poladian et al. 2009; Wilts et al. 2009), which are periodic dielectric structures in space composed of 2 or more media with different optical properties. These structures have a forbidden gap within which photons with certain energies cannot propagate and are completely reflected by the surface of the photonic crystal. The field has been developing at increasingly rapid pace since 1987, when structures (i.e., nanoarchitectures) with photonic band gaps were described (John 1987; Yablonovitch 1987).

An enormous variety of photonic structures can be found in nature. Butterflies, beetles (Ghiradella and Butler 2009; Seago et al. 2009; Shawkey et al. 2009a; Srinivasarao 1999; Biró et al. 2010), and even plants (Vigneron et al. 2005a; Glover and Whitney 2010) exhibit these kinds of structures. It has recently been shown that in certain species, such as the sexually dichromatic species *Hypolimnas bolina*, females prefer conspecific males that possess bright, iridescent blue/ultraviolet dorsal ornamentation (Kemp 2007) so that the "quality" of the photonic nanoarchitectures is well preserved from generation to generation. As emphasized by several recent reports, visual signals from conspecific individuals make important contributions to the mating behaviors of butterflies (Fordyce et al. 2002; Oliver et al. 2002; Bálint et al. 2012).

Several recent reviews have been published on photonic nanostructures of biological origin (Parker 2002; Vukusic and Sambles 2003; Fratzl 2007; Kinoshita 2008; Biró and Vigneron 2011). Butterflies are diverse and highly practical examples of these structures, as their color is generated by pigments and various nanostructures (Vukusic et al. 1999; Argyros et al. 2002; Kinoshita and Yoshioka 2005; Kertész et al. 2006a; Vértesy et al. 2006; Welch and Vigneron 2007; Vukusic and Stavenga 2009). The essentially flat wings of butterflies can be easily processed into convenient samples for both scientific examination and practical applications, such as solar cells (Zhang et al. 2009), sensors (Potyrailo et al. 2007; Biró et al. 2008), and many other uses (Huang et al. 2006; Berthier et al. 2007).

Lepidopteran wings, and the scales covering them, have a complex geometric structure on macroscopic- and microscopic-length scales. The membranes of the wings are generally covered with partially overlapping scales on both sides. Typical scale dimensions are in the range of 50–100 mm for length and 15–50 mm for width. Most species have 2 distinct layers of different scales, namely ground scales and cover scales. While the underside of the scale is rather featureless, the externally visible top surface usually exhibits a complex structure from the micron to the nanometer range. This top surface or the volume of the scale contains photonic nanostructures if the color of the wings is of structural origin. These nanostructures are mainly constructed of a chitinous matrix (the cuticle), including air holes (Scoble 1995; Kristensen 2003; Berthier 2006). These biological structures attract more

Downloaded From: https://complete.bioone.org/journals/Journal-of-Insect-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

and more attention because they constitute a transition between random and crystalline order (Shawkey et al. 2009; Liu et al. 2011). Recent papers reported that even a complete photonic band gap can be found in such partially ordered materials (Florescu et al. 2009).

It often may not be a straightforward task to determine if a certain butterfly exhibits structural color. Of course, transmission and scanning electron microscopy can reveal structural details, but these methods require time-consuming sample preparation (Shawkey et al. 2009b), expensive equipment, and physical destruction of the (potentially rare) exemplars. Sophisticated optical characterization (Kertész et al. 2006b; Wilts et al. 2009) can also reveal the presence of photonic nanoarchitectures, but the required instruments and the expertise may not be available in all laboratories; again, destructive sample preparation steps usually cannot be avoided. The method described by Mason (1923), based on soaking the specimen (sometimes for days) in liquids with a refractive index matching the refractive index of the nanoarchitecture, may also be an alternative. Dropping liquids (e.g., oil or ethanol) onto wings could identify nanostructures, but because these liquids cannot be found in the natural environments of butterflies, this method may raise concern from museum curators that it could damage the valuable samples. This type of method may induce persistent changes in the status of a specimen. Removing a few scales from the specimens, and investigating the single scales, could be also an alternative. Since these scales are relative small, this method requires more care and sophistication.

In this paper, a simple method based on cooling in ambient air to observe and investigate the color-change of the wings of several Lepidoptera species colored by photonic nanoarchitectures is presented in detail. Cooling the butterflies and allowing water vapor to condense quickly onto the micro- and nanostructures induces color change in their scales, which possess structural color arising from open nanoarchitectures. This simple method allows the observer to determine which colors are of structural origin, as pigment-colored wings do not show the color change observed in the case of structural colors. During the cooling experiments, significant differences were identified in a few species in the time lapse of the color change and how it changed; these differences originated from differences in air circulation in different nanoarchitectures. Some basic ideas that underlie the interaction of water with wings and the color-generating nanoarchitectures are highlighted. Recently, the heat capacity and thermal response of butterfly wings were studied (Pris et al. 2012). As softening in water vapor is the standard procedure for setting butterflies, water vapor condensation should not raise concerns from museum curators. It is well known that most Lepidoptera wings are hydrophobic (Wagner et al. 1996), which prevents liquid water from penetrating into the photonic nanostructure, because water droplets roll off the surface (Wagner et al. 1996), thus preventing the soaking of the wings in rain. Using the cooling method described herein, this difficulty can be easily avoided.

Materials and Methods

Eterusia taiwana (Wileman) (Lepidoptera: Zygaenidae) moths were obtained from the collections of the Laboratory of Natural Resource Conservation, Department of Biology and Institute of Life Science, National Sun Yat-Sen University, Taiwan. All other samples were obtained from the Lepidoptera

Downloaded From: https://complete.bioone.org/journals/Journal-of-Insect-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

collection of the Hungarian Natural History Museum.

For structural investigation of the wings of Lepidoptera species, scanning electron microscopy and transmission electron microscopy were used. Scanning electron microscopy samples were prepared by cutting off pieces of the wings, which were then attached to stubs with double-sided conductive tape. All samples were subsequently coated with 15 nm sputtered gold to allow for examination without charging. Cross-sectional transmission electron microscopy samples were prepared by embedding pieces of wings in special resin. Thin sections with a thickness of 70 nm were cut using an ultramicrotome and were transferred to copper grids.

For optical characterization, reflectance measurements were performed with an Avaspec 2048/2 fiber-optic spectrometer (Avantes, www.avantes.com). A coaxial illuminator/pick-up fiber was used under normal incidence conditions. Measurements were performed with unpolarized light using a combined halogen-deuterium light source. An Avaspec diffuse white standard was used as a reference sample. This system allowed accurate measurements in the 200–1000 nm wavelength range.

Measurements performed under controlled cooling were combined with spectroscopic observation of the color change. Three types of cooling apparatuses were used: (i) a custom-built Peltier cooler, which could be placed under the combined illuminator/pick-up fiber of the spectrometer; (ii) the deep freezer compartment of a commercially available refrigerator; and (iii) thin copper wires frozen into ice blocks within the deep freezer compartment. Peltier elements use the thermoelectric effect to directly convert electric power to a temperature difference. They are usually thin plates (3 mm in our experiments) and have two large sides (squares with 5 cm sides in our experiments) where the temperature difference can be created. Two Peltier elements in series were mounted on a CPU cooler fan for better heat dissipation. A shield was mounted around the Peltier elements to direct the airflow away from the samples and to ensure a uniform temperature. With this construction, a temperature of -5º C could be achieved in a normal room environment. Spectroscopic measurements were taken every second during the controlled cooling of the samples. An entire cooling cycle was typically completed over a time range of a few minutes. While Peltier coolers are not expected to be present in all laboratories working with butterflies, refrigerators are common in most of them. Therefore, some experiments were performed using the deep freezer compartment, cooling the butterflies for approximately 15 min.

Results

Color change in wings due to cooling

The initial motivation for this work was provided by the experimental observation of color changes in living *E. taiwana* moths. The moths were kept in a refrigerator at approximately 5° C to extend their lifespan. The cooled living *E. taiwana* moths exhibited color changes from green to dark brown to almost black when removed from the refrigerator. After being maintained at room temperature (23° C) for a few hours, a moth regained its former coloration. The experiment was repeated with dried museum samples, but no color changes were observed when the wings were positioned next to the bodies or on the bodies. The Peltier element was used to cool dried wing pieces that had been removed from the bodies, and color

Downloaded From: https://complete.bioone.org/journals/Journal-of-Insect-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

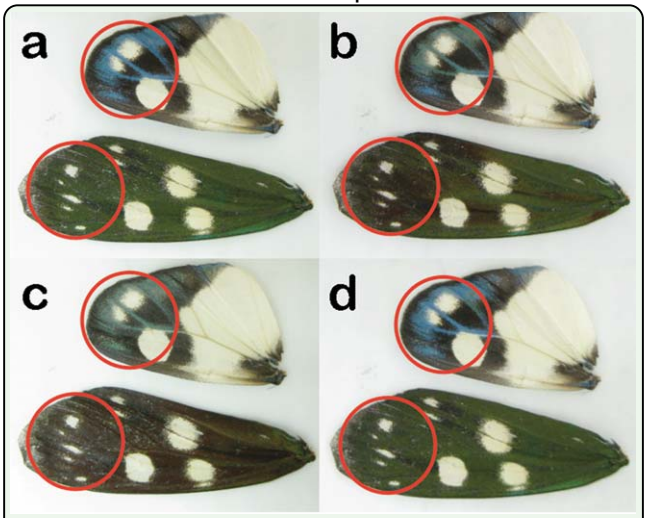


Figure 1. Color change on the wings of dried *Eterusia taiwana* due to water vapor condensation and freezing caused by cooling using the Peltier. Blue-black-white dorsal hindwing surface and green-white dorsal forewing surface a) at room temperature (23° C); b) after vapor condensation onto the structures (10° C); c) after freezing of the wings (-5° C); and d) after cooling and reheating to room temperature, when all of the water had evaporated from the wings. High quality figures are available online.

changes could be easily observed under an optical microscope (Figure 1). Water condensation (small water drops) and freezing (ice) could be easily observed on the surface of the Peltier cooler.

Further experiments were performed with wing samples from other dried Lepidoptera species with photonic nanostructures in their scales that were cooled with Peltier elements. From family Lycaenidae, *Albulina metallica* (C. and R. Felder) (blue dorsal wing surface), *Callophrys rubi* (L.) (green ventral wing surface), *Cyanophrys remus* (Hewitson) (blue dorsal and green ventral wing surfaces), and *Polyommatus daphnis* (Denis and Schiffermüller) (blue dorsal wing surface) were observed, and *Morpho aega* (Hubner) (blue dorsal wing surface) was observed from family Nymphalidae. As the width of the Peltier element was 5 cm, only small pieces of lepidopteran wings were used, in order to allow simultaneous cooling of several samples. Color changes could be easily observed on every sample (Figure 2).

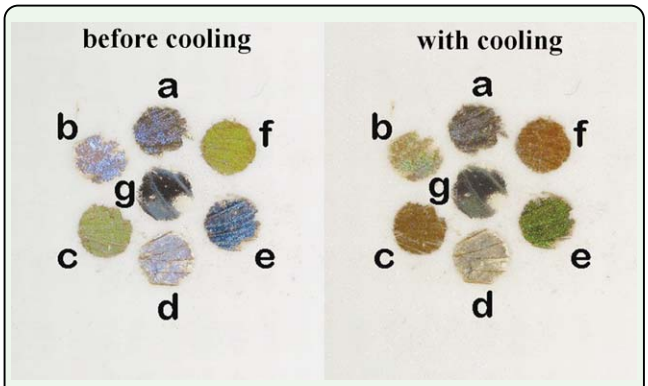


Figure 2. A simple display consisting of pieces of dried Lepidoptera wings before cooling and with cooling using the Peltier. Color changes can be observed due to water vapor condensation onto the photonic structures of the scales. The pieces of the wings are from: a) *Albulina metallica* (blue dorsal wing surface); b) *Morpho aega* (blue dorsal wing); c) *Cyanophrys remus* (green ventral wing); d) *Polyommatus daphnis* (blue dorsal wing); e) *Cyanophrys remus* (blue dorsal wing); f) *Callophrys rubi* (green ventral wing); and g) *Eterusia taiwana* (blue dorsal wing). High quality figures are available online.

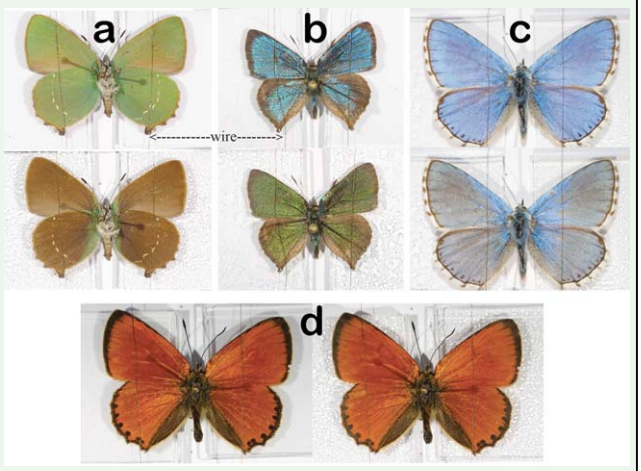


Figure 3. Lepidoptera species cooled in the refrigerator. a, b, and c are species with photonic structures in their scales, and d is a species without photonic structures in its scales. Color changes can be seen for species with photonic nanostructures, and the necessity of the heat sink can be observed near the bodies of a and c, where the color did not change, because the heat sinks were further from the bodies than in species b. Under identical conditions, the butterfly colored by pigments shown in d did not change color. a) *Callophrys rubi*, b) *Cyanophrys remus*, c) *Polyommatus bellargus*, d) *Lycaena virgaureae.* High quality figures are available online.

A simpler cooling method involves the use of a household refrigerator (cooling temperature: -10° C). Whole specimens can be cooled with a glass microscope slide under each wing as heat sink, allowing non-destructive measurements to be performed. Care has to be taken to

Downloaded From: https://complete.bioone.org/journals/Journal-of-Insect-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

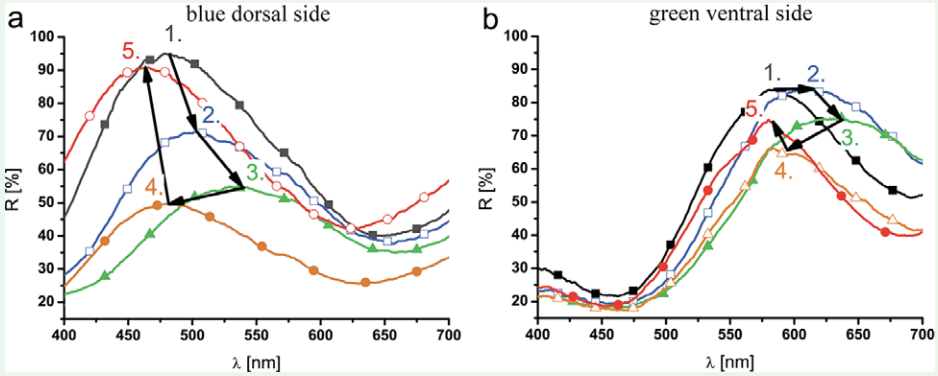


Figure 4. Spectral measurements on the surfaces of the wings of the moth *Eterusia taiwana*. The numbered curves show the stationary reflectance curves in various cooling stages: 1. dry status (23° C); 2. condensation (10° C); 3. freezing (-5° C); 4. melting (0° C); and 5. evaporation processes. High quality figures are available online.

provide good thermal contact between the microscope slide and the wing; a thin wire will suffice for this purpose, as shown in Figure 3. Four butterflies were mounted on a large substrate with glass plates under each wing. The samples were cooled for 30 min in the refrigerator before being removed to an ambient room environment. The necessity of the heat sinks can be observed on butterflies in Figure 3 a, c. Color changes cannot be observed near their bodies, where the wings are rapidly heated due to the absence of a cold thermal sink. As lepidopteran wings are not rigorously flat, some parts of the wings that are slightly closer to the cooled surface exhibit an earlier color change than others. A sample of the butterfly *Lycaena virgaureae* (L.) (Lepidoptera: Lycaenidae) was used for comparison (Figure 3d), as it is not colored by photonic nanoarchitectures, but by pigment (Vigneron et al. 2005b). It did not exhibit any color change upon cooling.

Spectroscopic measurements

E. taiwana wing samples were mounted on the surface of the cooling Peltier element, and spectroscopic measurements were performed *in situ* during cooling (Figure 4). The measurements began from a dry status (curve 1 in Figure 4). After applying the previously determined voltage to the Peltier elements, the temperature dropped below the dewpoint at approximately 10° C in a few minutes. When the dewpoint was reached and condensation of the water vapor began, the reflectance maximum shifted relatively rapidly to longer wavelengths (curve 2 in Figure 4), and the green/blue moths became dark-brown/green. The samples were maintained at that temperature range for approximately 5 min after the shift, but the reflectance spectrum did not change. Then, the temperature was set to below 0° C. The reflectance maximum shifted rapidly and further to longer wavelengths (curve 3 in Figure 4), when the wing pieces became completely frozen. After waiting 5 min, during which time the position of the reflectance maximum did not change, the applied voltage was turned off to allow the samples heat up to ambient temperature (23° C). When melting began, the reflectance maximum shifted to shorter wavelengths with another rapid progress (from curve 3 to curve 4 in Figure 4) and stopped for approximately 1 min when the ice in the wings was completely melted (0° C). In a similar process, when the water in the samples began to evaporate, the reflectance maximum shifted towards shorter wavelengths and increased further in intensity until a color close to the original color was achieved. Full restoration of the wing color was accomplished by drying overnight, indicating that a certain small but measurable fraction of water penetrated into

Downloaded From: https://complete.bioone.org/journals/Journal-of-Insect-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

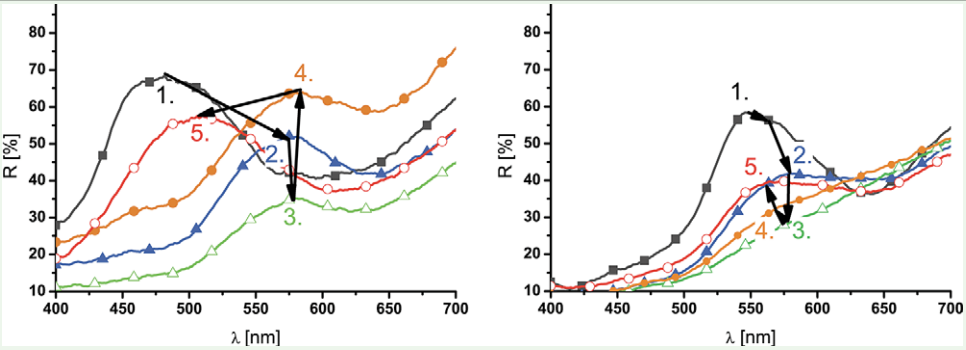


Figure 5. Spectral measurements on the surfaces of the wings of the butterfly *Cyanophrys remus*. The numbered curves show the stationary reflectance curves in various stages: 1. dry status (23° C); 2. condensation (10° C); 3. freezing (-5° C); 4. melting (0° C); and 5. evaporation processes. High quality figures are available online.

the cuticle. When measuring the change of the reflection spectrum during a cooling cycle for butterflies with structural coloration on both the dorsal and ventral wing side, quite different behavior could be observed on the same wing, but on a different side of the wing (dorsal vs. ventral). Similar spectroscopic measurements were performed on several other lepidopteran species, such as the well-characterized species *C. remus* (Kertész et al. 2006b) (Figure 5). During cooling, the green ventral side of this species became brown. However, a very different shift of the reflection spectrum was found on this species compared to the *E. taiwana* species. The temperature of the Peltier cooler and the shift of the reflection maximum can be seen for the green ventral side of *A. metallica* in Figure 1.

Further experiments were performed on several species to investigate the time dependence of the color change (see Figure 2 for an example). The samples were mounted on the Peltier cooler and cooled to approximately 10° C, the temperature at which condensation can begin. After 300–400 sec, the cooling apparatus was turned off, and the samples were allowed to warm up to ambient temperature (23° C). The time-dependent shift of the reflectance peaks was investigated. The resulting curves depended on the complex structural differences between the wing scale nanoarchitectures and vapor condensation rates. Further investigation with more precise control of environmental conditions will be required to obtain additional insight into the processes taking place.

Repeated cooling-heating cycles were used to examine the long-term behavior of the spectral shift. The samples were cooled (to approximately 10° C), and after the reflectance maximum had not changed for 5 min, the samples were heated to ambient temperature (23° C); following an additional 5 min, the whole cycle was repeated. After approximately the third cycle, the butterflies completely lost their color. Furthermore, cuticles absorbed water, and the wings became malleable. Drying the samples for a few days fully restored the initial conditions of the wings. Observation of wing softening is not surprising, as this is the common preparation procedure for fixing the position of museum specimens.

Local cooling by heat sink

This cooling method can also be used to change the color of lepidopteran wings locally. Cooling wings in specified small areas makes it possible to "write" on the wing surface. One method for this involves the use of a matrix built from miniature Peltier cells (each cell is used as a pixel), but a simpler method

Downloaded From: https://complete.bioone.org/journals/Journal-of-Insect-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

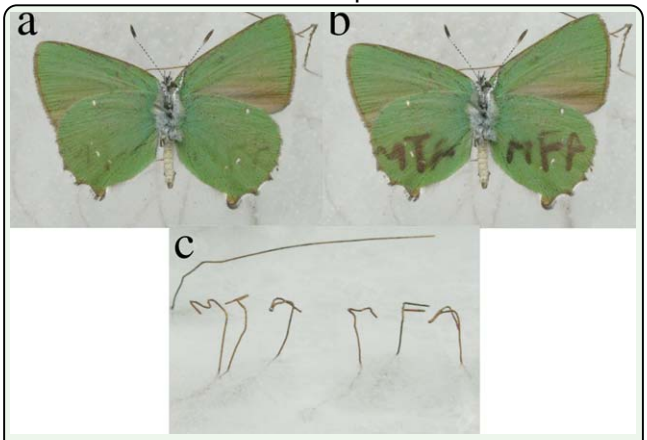


Figure 6. *Callophrys rubi* was placed onto cooled wires that were used to form characters. One end of the wires was frozen into ice, and the other end was used to form characters (c). The cold wires cool the surrounding area, where the condensation can begin. The images were collected 2-3 sec (a) and 1 min (b) after putting the samples onto the wires. High quality figures are available online.

involves using cold wires below the wings. A cold wire cools a small area around it. If the temperature around the wire is lower than the dewpoint, condensation begins and changes the color of the wing.

In our experiments, one end of the thin copper wires was frozen into ice blocks, while the other end was used to form horizontal characters above the ice blocks. The butterfly *C. rubi* was placed onto these characters, and the characters were shaped to provide the best thermal contact with the wing membrane (Figure 6). Copper has very good heat conductivity; it can easily transfer heat from the wings to the ice blocks, keeping the area cool around the wires on the wings. The temperature in the cooled area can be set by adjusting the distance between the ice and the characters. If the temperature of the wires is sufficiently high to be close to the dewpoint, the colored area around them will be narrower. If the wing is too close to the ice block, the temperature of the entire wing can be lower than the dewpoint; this causes the entire wing to change color. The colored area around the wires widens in time as water vapor condenses. The writing begins to appear a few seconds after placing the butterfly onto the wires, and it can be clearly observed after waiting another minute.

Discussion

Role of the heat sink in the color change

The difference between the dried and living *E. taiwana* moths in our first observations was due to the bodies of the living specimens having higher heat capacities due to their water contents, making the bodies act as heat sinks. In a resting position, these moths keep their closed wings near their bodies, unlike many butterflies. The dried museum specimens have their wings positioned on the sides of their bodies, as is usual for museum exemplars. Additionally, museum specimens have lost most of the water content present in the living insect. Thus, they have a low heat capacity, so they cannot provide an adequate heat sink; the wings would need to be heated to room temperature (23° C) before significant vapor condensation could take place. This might be the reason that these specimens did not exhibit any color change after being removed from the deep freezer compartment of the refrigerator (cooling temperature: -10° C). If a large heat sink is provided by mounting microscope slides under the wings to increase the heat capacity of the combined system (wing + microscope slide), the wings can be kept cool for a sufficient amount of time to allow condensation to take place.

Investigation of the possible biological significance of the color changes of wings (when cooled below the dewpoint in an ambient atmosphere) with respect to the survival strategy of these species might be a very interesting subject for visual ecology, but it is outside the scope of this paper.

Downloaded From: https://complete.bioone.org/journals/Journal-of-Insect-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

Water penetration and wing softening

As a cuticle absorbs water, it swells, thus altering the thickness of small structures made of cuticle. This swelling effect can be observed in Figures 4 and 5, where the reflection curves do not immediately return to the initial position, and the maxima have lower intensities after the cooling-heating cycle. However, overnight drying will fully recover the initial shape and position of the reflection curves. Because total swelling of the structures can be achieved by several cooling cycles (usually more than 3), it is important that the reflection variations of several species are compared in the first cooling cycle to minimize the swelling effect.

When preparing museum specimens, butterflies are usually softened in saturated water vapors for hours or days to cause them to become malleable so they can be set. This is a slow process, as water vapors have to penetrate into the volume of the specimen aided by daily temperature variations. Although a sample absorbs significant amounts of water, in most cases, no liquid water will be present on its surface. A relaxed status of the wings is achieved from the repeated cooling-heating cycles within a few minutes instead of the days required for saturated water vapors.

Color change and intensity variations in spectroscopic measurements

The photonic crystal structures in lepidopteran wings are mainly constructed from cuticle and air. Cuticle has a moderate refractive index (n = 1.56) (Vukusic et al. 1999). The position of the reflectance maximum (λ_{max}) can be easily estimated with a simple equation: $\lambda_{max} = n_{eff}*d/m$ (Vigneron et al. 2006), where n_{eff} is the average refractive index, d is the thickness of one period in a periodic structure, and m is an integer. When air (n = 1) is replaced with water (n = 1.33), the contrast of the refractive indices decreases, which in turn will modify the average refractive index value. This decrease of the refractive index contrast shifts the stop-band (i.e., the reflectance maximum) to higher wavelengths, as can be observed in the spectral measurements presented in Figures 4 and 5 between the curves labeled 1 and 2 The reverse process can also be observed between the curves labeled 4 and 5. Freezing the samples generated quite different results. For *C. remus,* the position of the reflectance maximum was changed only moderately by freezing, while for *E. taiwana,* a clear spectral shift occurred. There were also differences for the same species exhibiting different color on the two side of the wing, when measuring on the dorsal or ventral side of the wings. Structural differences are likely responsible for these different behaviors, as will be discussed below in the context of the scanning electron microscopy figures.

The change in the intensity of the reflectance maximum during the cooling experiments depended on various parameters, such as the modifications induced in the surface geometry of the wing by wetting and freezing, the deformation of the chitinous structures due to water absorption, and the presence of water and ice on top of the scales. All of these parameters could have differed from individual to individual. Due to this complex behavior, a precise interpretation of the observed variations in intensity is beyond the scope of this paper.

Effect of the nanoarchitecture during cooling

During the simultaneous cooling experiments with the Peltier cooler (Figure 2), it was observed that the wings of different Lepidoptera species exhibited slight differences in the time elapsed from the initiation of cooling until color changes were clearly observed. This can

Downloaded From: https://complete.bioone.org/journals/Journal-of-Insect-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

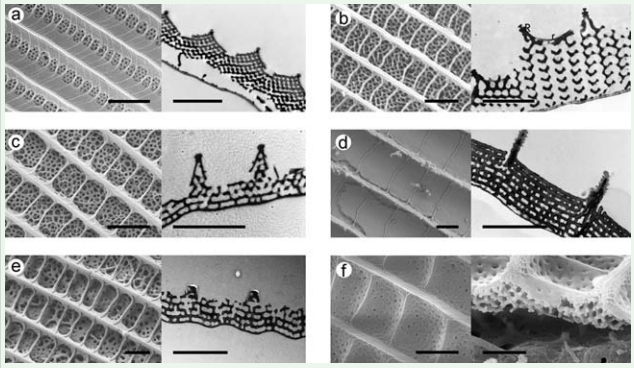


Figure 7. Scanning electron microscopy (SEM)/transmission electron microscopy (TEM) images of the butterflies. Left: SEM; right: TEM, except for f, where both are SEM images. All scale bars are 2 mm. a) *Cyanophrys remus* (blue dorsal wing surface); b) *Callophrys rubi* (green dorsal wing surface, TEM image is reprinted from Ghiradella and Radigan 1976); c) *Polyommatus daphnis* (blue dorsal wing surface); d) *Chrysiridia ripheus* (blue-green ventral wing surface); e) *Albulina metallica* (blue dorsal wing surface); f) *Eterusia taiwana* (green dorsal wing surface). High quality figures are available online.

be explained by taking into account the differences in the color-generating nanoarchitectures in the scales.

If the surface temperature drops below the dew point, condensation begins on the surface, and it is quite difficult to calculate the exact condensation rate. The diffusion and heat equations have to be solved in parallel, making the boundary conditions match (for example, see Kandlikar 1999). We will not attempt to solve this problem, but we will attempt to highlight some essential features of the process. Lepidoptera wings are quite complex structures, possessing a wing membrane and usually several layers of scales. Color is measured in the upper or observed side of the wing, and cooling is applied to the lower or cooled side. The upper side could be dorsal or ventral, depending on which side is being investigated. The transportation of heat from the observed scales to the cooled scales is quite complex. Therefore, the interactions between different scales and between the scales and the membrane have to be taken into account. As the observation of color change occurs on the upper part of the wing, we attempt to restrict our interpretation to this part. When water vapor begins to condense, it will decrease the relative humidity below the critical value; for further condensation, a new supply of wet air is required. The more open the structure is, the more easily the supply of wet air will penetrate it. The fastest color changes were observed for *C. remus*, *P. daphnis*, and *C. rubi*, which have similar nanoarchitectures, while slightly slower color modifications were observed for *A. metallica* and *E. taiwana*. The scales of *E. taiwana* exhibit a quite different, more closed structure compared to the other species examined in this work; the surfaces of their scales have fewer and smaller holes than the scales of the investigated butterflies (Figure 7). It is possible that in closed nanoarchitectures, such as those of *Chrysiridia ripheus*, the supply of wet air will be insufficient for color changes to occur (Figure 7d). The cooling experiments demonstrated this to be the case; no significant color change could be observed in this species.

Conclusions

Our investigation of the rapid cooling of butterfly and moth wings in ambient air demonstrated that the wings of these species, which have photonic crystal-type nanoarchitectures with open structures, exhibited clear changes in coloration. Our data show that the durations and magnitudes of the color changes and the ways in which they occurred were specific for certain structures (i.e., species), but small variations may also have occurred from individual to individual. In particular, in some closed photonic nanoarchitectures, such as those of the moth *C. ripheus,* a rapid color change was not observed, because water vapor cannot easily penetrate such closed structures. Therefore, if color change is observed on cooling, this is a clear indication that the scales of the examined butterfly and moth species are colored by photonic nanoarchitec-

Downloaded From: https://complete.bioone.org/journals/Journal-of-Insect-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

tures, while the absence of the color change cannot be interpreted as unambiguous evidence that the scales are colored only by pigments. In these cases, detailed structural (i.e., scanning electron microscopy and transmission electron microscopy) and spectral investigation may be required. Nevertheless, this quick and simple test can prove very useful in the rapid screening of species to determine whether it may be worth performing the time-consuming and expensive high-resolution characterization procedures. At the same time, this screen provides information regarding the open (*C. remus, C. rubi*) or more closed (*E. taiwana, A. metallica, C. ripheus*) characters of the structures generating the structural colors.

Cooling can be achieved using the deep freezer compartment of a common refrigerator if a large heat capacity heat sink is mounted in good thermal contact with the wings. This makes large-scale application of the method possible. It is worth pointing out that well-dried collection specimens will not exhibit a color change without an appropriate heat sink under their wings. Another important advantage of cooling in the refrigerator may be that it is non-destructive; even rare exemplars could be examined in this way without risking damage to the samples.

If repeated cooling and heating cycles are performed in succession, the wings can be softened in minutes. This rapid softening is attributed to repeated water vapor condensation taking place in the interior of the micro- and nanostructures of the scales and of the wing membranes. This will render the wings malleable without destruction of the exemplars or the need to keep the butterflies in saturated water vapors for several days.

If local cooling is provided, such as by thin copper wires, it is possible to "write" on wings possessing structural colors. This color change effect upon water vapor condensation could be used to construct a tuneable photonic crystal-based flat panel display. The color change can be induced by the local condensation of water vapors, which are always present in ambient air. Each pixel should be mounted on a miniature Peltier element, and the temperature (and color) could be set with active feedback. The similar process of capillary condensation has been employed for optical switching in porous optical superlattices (Barthelemy et al. 2007) and is used by the beetle *Dynastes hercules* to reversibly switch color depending on the relative humidity of the environment (Rassart et al. 2008).

Acknowledgements

The work in Hungary was supported by OTKA grant PD83483. The collaboration of Hungarian and Taiwanese scientists was made possible by a joint agreement between the Hungarian Academy of Science and the NSC in Taiwan for mobility. K. Kertész gratefully acknowledges financial support from the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

References

Argyros A, Manos S, Mckenzie DR, Cox GC, Dwarte DM. 2002. Electron tomography and computer visualisation of a three-dimensional `photonic' crystal in a butterfly wing-scale. *Micron* 33: 483–487.

Bálint Z, Kertész K, Piszter G, Vértesy Z, Biró LP. 2012. The well-tuned blues: The role of structural colours as optical signals in the species recognition of a local butterfly fauna (Lepidoptera: Lycaenidae: Polyommatinae),

Downloaded From: https://complete.bioone.org/journals/Journal-of-Insect-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

Journal of the Royal Society Interface 9: 1745–1756.

Barthelemy P, Ghulinyan M, Gaburro Z, Toninelli C, Pavesi L, Wiersma DS. 2007. Optical switching by capillary condensation. *Nature Photonics* 1: 172–175.

Berthier S. 2006. *Iridescences: The Physical Colors of Insects*, 1st edition. Springer.

Berthier S, Boulenguez J, Bálint Z. 2007 Multiscaled polarization effects in *Suneve coronata* (Lepidoptera) and other insects: application to anti-counterfeiting of banknotes. *Applied Physics A: Materials Science and Processing* 86: 123–130.

Biró LP, Kertész K, Vértesy Z, Bálint Z. 2008. Photonic nanoarchitectures occurring in butterfly scales as selective gas/vapor sensors. In: Creath K, Editor. *The Nature of Light: Light in Nature II*, *Proceedings of SPIE* 7057: 705706

Biró LP, Kertész K, Horváth E, Márk GI, Molnár G, Vértesy Z, Tsai J-F, Kun A, Bálint Z, Vigneron J-P. 2010. Bioinspired artificial photonic nanoarchitecture using the elytron of the beetle *trigonophorus rothschildi varians* as a 'blueprint'. *Journal of the Royal Society Interface* 7: 887–894.

Biró LP, Vigneron JP. 2011. Photonic nanoarchitectures in butterflies and beetles: valuable sources for bioinspiration. *Laser and Photonics Review* 5(1): 27-51.

Florescu M, Torquato S, Steinhardt PJ. 2009. Designer disordered materials with large, complete photonic band gaps. *Proceedings of the National Academy of Sciences USA* 106: 20658–20663.

Fordyce JA, Nice CC, Forister ML, Shapiro AM. 2002. The significance of wing pattern diversity in the Lycaenidae: mate discrimination by two recently diverged species. *Journal of Evolutionary Biology* 15: 871–879.

Fratzl P. 2007. Biomimetic materials research: what can we really learn from nature's structural materials? *Journal of the Royal Society Interface* 4: 637–642.

Ghiradella HT, Radigan W. 1976. Development of butterfly scales: II. Struts lattices and surface tension. *Journal of Morphology*150: 279–298.

Ghiradella HT, Butler MW. 2009. Many variations on a few themes: a broader look at development of iridescent scales (and feathers). *Journal of the Royal Society Interface* 6: S243–S251.

Glover BJ, Whitney HM. 2010. Structural colour and iridescence in plants: the poorly studied relations of pigment colour. *Annals of Botany* 105(4): 505–511.

Huang J, Wang X, Wang ZL. 2006. Controlled Replication of Butterfly Wings for Achieving Tunable Photonic Properties. *Nano Letters* 6(10): 2325–2331.

Joannopoulos JD, Meade RD, Winn JN. 2008. *Photonic Crystals: Molding the Flow of Light*, 2nd edition. Princeton University Press.

John S. 1987. Strong localization of photons in certain disordered dielectric superlattices. *Physical Review Letters* 58: 2486–2489.

Kandlikar SG. 1999. *Handbook of Phase Change: Boiling and Condensation*. Taylor and Francis.

Downloaded From: https://complete.bioone.org/journals/Journal-of-Insect-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

Kemp DJ. 2007. Female butterflies prefer males bearing bright iridescent ornamentation. *Proceedings of the Royal Society B: Biological Sciences* 274: 1043–1047.

Kertész K, Bálint Z, Vértesy Z, Márk GI, Lousse V, Vigneron JP, Biró LP. 2006a. Photonic crystal type structures of biological origin: Structural and spectral characterization. *Current Applied Physics* 6: 252–258.

Kertész K, Bálint Z, Vértesy Z, Márk GI, Lousse V, Vigneron JP, Rassart M, Biró LP. 2006b. Gleaming and dull surface textures from photonic-crystal-type nanostructures in the butterfly Cyanophrys remus. *Physical Review E* 74: 021922.

Kinoshita S, Yoshioka S. 2005. Structural colors in nature: the role of regularity and irregularity in the structure. *Computer Physics Communications* 6: 1442–1459.

Kinoshita S. 2008. *Structural Color in the Realm of Nature*. World Scientific.

Kristensen NP. 2003. Lepidoptera, Moths and Butterflies: Morphology, Physiology and Development, Volume 2. In: *Handbook of Zoology,* Volume 4, Part 36. Walter de Gruyter.

Liu F, Dong B, Zhao F, Hu X, Liu X, Zi J. 2011. Ultranegative angular dispersion of diffraction in quasiordered biophotonic structures. *Optics Express* 19: 7750–7755.

Mason CW. 1923. Structural Colors in Feathers. *Journal of Physical Chemistry* 27(3): 201–251.

Nijhout HF. 1991. *The development and evolution of butterfly wing patterns*. Smithsonian Institution Press

Oliver JC, Robertson KA, Monteiro A. 2002. The significance of wing pattern diversity in the Lycaenidae: mate discrimination by two recently diverged species. *Journal of Evolutionary Biology* 15: 2369–2375.

Parker AR. 2002. Natural Photonic Engineers. *Materials Today* 5(9): 26–31.

Poladian L, Wickham S, Lee K, Large MCJ. 2009. Iridescence from photonic crystals and its supression in butterfly scales. *Journal of the Royal Society Interface* 6: S233–S242.

Potyrailo RA, Ghiradella H, Vertiatchikh A, Dovidenko K, Cournoyer JR, Olson E. 2007. Morpho butterfly wing scales demonstrate highly selective vapour response. *Nature Photonics* 1: 123–128.

Pris AD, Utturkar Y, Surman C, Morris WG, Vert A, Zalyubovskiy S, Deng T, Ghiradella HT, Potyrailo RA. 2012. Towards high-speed imaging of infrared photons with bio-inspired nanoarchitectures. *Nature Photonics* 6: 195–200.

Rassart M, Colomer JF, Tabarrant T, Vigneron JP. 2008. Diffractive hygrochromic effect in the cuticle of the hercules beetle *Dynastes hercules*. *New Journal of Physics* 10: 033014.

Scoble MJ. 1995. *The Lepidoptera: form, function, and diversity*, 2nd ednition. Oxford University Press.

Seago AE, Brady P, Vigneron JP, Schultz TD. 2009. Gold bugs and beyond: a review of iridescence and structural colour mechanisms

Downloaded From: https://complete.bioone.org/journals/Journal-of-Insect-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

in beetles (Coleoptera). *Journal of the Royal Society Interface* 6: S165–S184.

Shawkey MD, Morehouse NI, Vukusic P. 2009a. A protean palette: colour materials and mixing in birds and butterflies. *Journal of the Royal Society Interface* 6: S221–S231.

Shawkey MD, Saranathan V, Palsdottir H, Crum J, Ellisman MH, Auer M, Prum RO. 2009b. Electron tomography, three-dimensional Fourier analysis and colour prediction of a three-dimensional amorphous biophotonic nanostructure. *Journal of the Royal Society Interface* 6: S213–S220.

Srinivasarao M. 1999. Nano-Optics in the Biological Word: Beetles, Butterflies, Birds and Moths. *Chemical Reviews* 99: 1935–1961.

Vértesy Z, Bálint Z, Kertész K, Vigneron JP, Lousse V, Biró LP. 2006. Wing scale microstructures and nanostructures in butterflies--natural photonic crystals. *Journal Of Microscopy* 224: 108–110.

Vigneron JP, Rasart M, Vértesy Z, Kertész K, Sarazzin M, Biró LP, Ertz D, Lousse V. 2005a. Optical structure and function of the white filamentary hair covering the edelweiss bracts. *Physical Review E* 71: 011906.

Vigneron JP, Lousse V, Biró LP, Vértesy Z, Bálint Z. 2005b. Reflectance of topologically disordered photonic-crystal films. In: Adibi A, Lin SY, Scherer A, Editors. *Photonic crystal materials and devices III*, *Proceedings of SPIE* 5733: 308–315.

Vigneron JP, Rassart M, Vandenbem C, Lousse V, Deparis O, Biró LP, Dedouaire D, Cornet A, Defrance P. 2006. Spectral filtering of visible light by the cuticle of metallic woodboring beetles and microfabrication of a matching bioinspired material. *Physical Review E* 73: 041905-1.

Vukusic P, Sambles JR, Lawrence CR, Wootton RJ. 1999. Quantified interference and diffraction in single Morpho butterfly scales. *Proceedings of the Royal Society B: Biological Sciences* 266: 1403–1411.

Vukusic P, Sambles JR. 2003. Photonic structures in biology. *Nature* 424: 852–855.

Vukusic P, Stavenga DG. 2009. Physical methods for investigating structural colours in biological systems. *Journal of the Royal Society Interface* 6: S133–S148.

Wagner T, Neinhuis C, Barthlott W. 1996. Wettability and Contaminability of Insect Wings as a Function of Their Surface Sculptures. *Acta Zoologica* 77: 213–225.

Welch VL, Vigneron JP. 2007. Beyond butterflies – the diversity of biological photonic crystals. *Optical and Quantum Electronics* 39: 295–303.

Wilts BD, Leertouwer HL, Stavenga DG. 2009. Imaging scatterometry and microspectrophotometry of lycaenid butterfly wing scales with perforated multilayers. *Journal of the Royal Society Interface* 6: S185–S192.

Yablonovitch E. 1987. Inhibited Spontaneous Emission in Solid-State Physics and Electronics. *Physical Review Letters* 20: 2059–2062.

Zhang W, Zhang D, Fan T, Gu J, Ding J, Wang H, Guo Q, Ogawa H. 2009. Novel Photoanode Structure Templated from Butterfly Wing Scales. *Chemistry of Materials* 21: 33–40.

Downloaded From: https://complete.bioone.org/journals/Journal-of-Insect-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

Zollinger H. 2003. *Color Chemistry: Syntheses, Properties and Applications of Organic Dyes and Pigments*, 3rd revised edition. Wiley.

Downloaded From: https://complete.bioone.org/journals/Journal-of-Insect-Science on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use