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# Large-Scale Artificial Production of Coleoptera Cuticle Iridescence and Its Use in Conformal Biodegradable Coatings

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**In this study, bioinspired chitinous manufacturing is leveraged to artificially reproduce iridescent gratings found in the cuticles of beetles living in concealed environments. Combining this with a melanin-like background achieves a vibrant iridescence, similar to that produced by the multilayer reflectors in leaf beetles. In this process, the controlled production of optical structures is allowed using chitinous polymers, the same components that produce color in arthropod cuticles. This not only aids in understanding the functionality and evolutionary advantages of these structures without being limited to the existing solutions in animals—which entangle the solutions for several problems with the rubble of past functionalities—but also enables the creation of color with the second most abundant organic material on Earth, only surpassed in its ubiquity by cellulose. However, unlike cellulose-based materials, the chitinous optical structures are produced by avoiding the use of strong organic solvents, representing a simplified approach to producing vibrant, iridescent, fully biodegradable, and ecologically integrated colors. Herein, the production of hundreds of square centimeters of iridescent chitinous surfaces, a scale suitable for imparting biodegradable color to large 3D objects, is demonstrated.**

## 1. Introduction

Bioinspired engineering seeks to use, at the product level, information and solutions developed by biological systems through millions of years of evolution.[1,2] Beyond the mere production of engineering solutions, there are also essential lessons for humanity's sustainable development regarding how organisms integrate their manufacturing within circular ecosystems while under the strict need to minimize energy and resource use. Thus, in the last decade, the field of bioinspired engineering has expanded from the adaptation of biological solutions using artificial materials to bioinspired manufacturing—a paradigm that is centered on the use of unmodified natural molecules with their native designs and inner synergies and that has given rise to an era-defining production model that can be seamlessly integrated into the surrounding ecosystem.[3] An exemplar of this development is the rapid growth of biomimetic manufacturing using chitin—the second most abundant organic molecule on Earth—which has, in just a decade, transitioned from millimeter-size samples that reproduce the synergies and mechanical characteristics of insect cuticles[4] to meter-scale products weighing tens of kilograms.[5] The uniquely rapid development of bioinspired chitinous manufacturing is due to a positive feedback loop between advances in the biological and artificial manufacturing realms (**Figure 1**a). While we learn from natural systems how to manufacture more complex products and functionalities using unmodified chitinous molecules, we also gain the ability to replicate, with increasing detail, biological structures using their native components. The latter translates into accurate models that can be used to reverse engineer the chitinous structures of organisms by reproducing each of their features in isolation, allowing their multiple roles to be disentangled and providing new information to improve our use of chitin in product manufacturing.[4,6]

While chitinous polymers are mainly used in biomimetic manufacturing for their mechanical characteristics, they often have other roles in natural systems, and the production of structural color in arthropods—specifically on the elytra of beetles (Coleoptera)—is one such an exceptionally eye-catching illustration of this.[7–9] In contrast to mere pigmentation, structural color—in addition to being highly saturated and fade proof—offers

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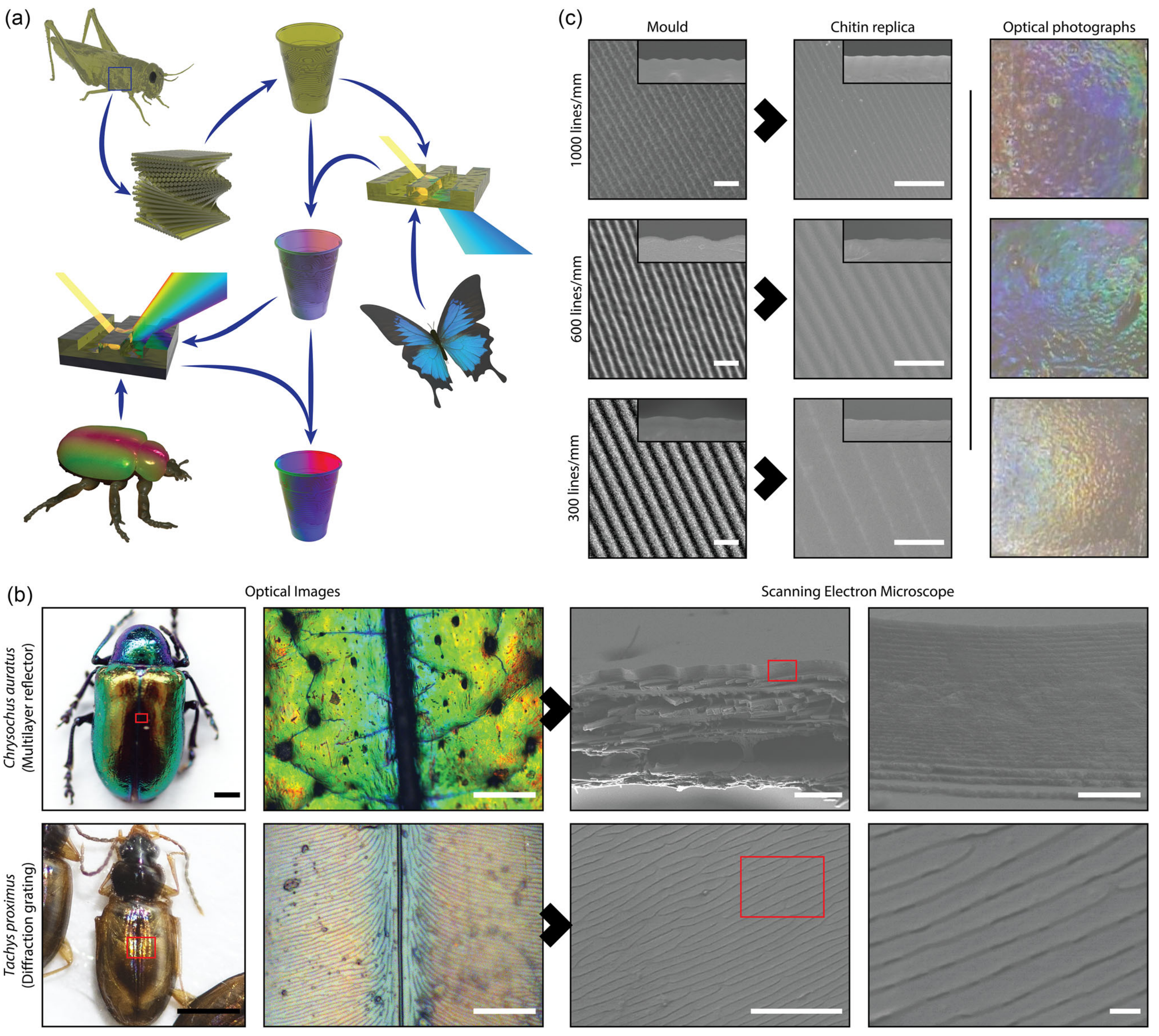


**Figure 1.** Biomimetic manufacturing of chitinous color. a) A positive feedback loop between the fields of arthropod physiology and bioinspired manufacturing. The first uses of unmodified chitinous polymers in product manufacturing[41] resulted from replicating part of their native hierarchical design,[4] giving rise to synergies and mechanical properties that are exhibited by the material in the arthropod cuticle. This new ability to manufacture chitin in complex forms enabled, among other applications, the reproduction in isolation of butterfly scales' ridges, shedding light on the correlation between their height and the hue of the animals' wings and enabling the first artificial production of chitinous structural color.[6] Here, we use this new capability to produce the structural chitinous color with melanin in beetles, isolating the role of cuticular gratings to create vibrant iridescent colors. b) Images at different magnifications of the cuticles of two beetles producing iridescence by two different mechanisms: a dogbane beetle (*Chrysochus auratus,* multilayer reflector, top row) and a riverbank ground beetle (*Tachys proximus*, diffraction grating, bottom row). From left to right: photographs of the beetles (scale bars are 1.5 mm); microscope images of the elytra surfaces in the areas marked with rectangles in the first column (scale bars are 150 μm); scanning electron microscope (SEM) images of the elytron cross-section for *C. auratus* and the surface for *T. proximus* (scale bars are 20 μm); and SEM images of the areas marked with rectangles in the third column, showing the lamellae acting as a multilayer reflector for *C. auratus* and the topography acting as a diffraction grating for *T. proximus* (scale bars are 2 μm). c) Production of diffraction gratings and iridescence in the otherwise-transparent chitinous films. The first column shows SEM images (scale bars are 5 μm) of the negative molds used to cast a chitinous solution and produce structured films. Three different ridge distances (3.3, 1.6, and 1 μm) were tested. The latter produced the strongest iridescence effect of the three, despite the average height between the lowest and highest points of the trenches being only 76 nm in a film 36 μm thick. Insets are 6.2 μm long cross sections of the films. The rightmost column contains optical images of the films without backgrounds.

additional functionalities, like angle-dependent reflectance (i.e., iridescence) and polarization.[10–13]Iridescence is particularly striking because a single nanostructure can produce colors across the entire visible spectrum as the observer's position changes, which is a feature that plays critical biological roles in animals, such as communicating positional, sex, and mating information[14,15] or—remarkably successfully—confounding visually hunting predators.[16]

The most common nanostructures that beetles use to produce iridescent and metallic colors are multilayer reflectors in their cuticles that are composed of alternating layers of chitin and melanin (for an in-depth review of structural color and iridescence in beetles, see ref. [17]). Several beetles also have 2D and 3D biophotonic crystals within their setae and scales,[9,18] but some species of beetles have evolved iridescent diffraction gratings on the outermost layer of their cuticles (i.e., epicuticles).[17] This last approach predominates among dark-colored beetles living in concealed (i.e., dark) and damp habitats and produces less conspicuous results than the striking iridescence created by the multilayer reflectors of other beetles (e.g., leaf beetles; Figure 1b and S1, Supporting Information).[17,19] The fact that gratings on the beetles' cuticles significantly impact their wettability and surface friction in moist environments[20] suggests that iridescence, when produced by cuticular gratings in dark habitats, could be simply a byproduct of selection for nonoptical properties.[19]

In this study, we use recent advances in micro- and nanostructuring of chitinous polymers to reproduce, in a controlled manner, the iridescence of a beetle's chitinous diffraction grating. However, we also seek to move beyond the animal kingdom's achievements and attempt to produce vibrant iridescent colors by optimizing those gratings—a route to achieving such optical effects that is underexplored by evolution compared to the optimization of the multilayer reflectors typically observed in leaf beetles (Chrysomelidae).[17] Toward this end, we include a dark pigment similar to melanin underneath the grating to increase the saturation of the diffracted colors.[21,22] This approach, at the interface of animal morphology and sustainable manufacturing, enables the controlled creation of optical structures using chitinous polymers, the same components responsible for color in arthropod cuticles. The artificial chitinous optical structures deepen our understanding of the functionality and evolutionary benefits of these structures in nature and pave the way for color creation using chitin, the second most ubiquitous organic material on Earth, surpassed only in abundance by cellulose. We specifically demonstrate 1) that minimal variations in the chitinous grating structures of beetles living in concealed habitats can have dramatic optical effects, transforming previously timid reflections into vibrant and eye-catching iridescence and supporting the hypothesis that iridescence in that case is a byproduct and not a driver of evolution and 2) that strong iridescent effects can be achieved in chitinous polymers with a simple surface grating and an underlying light-absorbent background instead of the complex multilayered reflector commonly found in arthropods. This second point offers a novel method for producing large-scale chitinous structural color, demonstrating the production of hundreds of square centimeters of iridescent chitinous surfaces, a scale suitable for imparting biodegradable color to large 3D objects. This marks a significant leap in the scale of chitinous structural color production, from a few square micrometers using nanoimprinting[6] to tens of centimeters using casting. This is a staggering increase of 10 quadrillion times or sixteen orders of magnitude with a process that, unlike cellulose, does not require strong organic solvents for its dissolution and a molecule with demonstrated circularity, environmentally benign sourcing, and ecological integration.[23,24]

## 2. Production of Chitinous Gratings

In our study, we first explore chitinous structural color by evaluating the role of grating spacing in color production. Because diffraction gratings are standard components of optical systems for tasks like wavelength selection, beam splitting, and beam shaping, they are commercially available at low cost, enabling the exploration of different characteristics at macroscopic scales. We examine variations within the common range of dimensions reproducible at a large scale and found in the diffraction gratings of coleopteran cuticles: periodicities between 1.0 and 3.3 μm (1000–300 lines $mm^{-1}$; Figure 1c), based on observations of different animals that suggest that 1 μm spacing are the approximate threshold separating iridescent (below 1 μm) and non-iridescent (above 1 μm) species.[20] The fabrication of the biomimetic chitinous gratings follows our previous results for producing freestanding nano- and micrometric topographies using chitinous polymers.[25,26] Briefly, we use soft lithography to produce negative polydimethylsiloxane copies of the optical gratings, which are then replicated by solvent-casting on chitosan extracted from the wasted shells of industrially processed tiger prawns (*Penaeus monodon*) and dispersed at a 1% concentration in a 1% aqueous solution of acetic acid (for comparison, table vinegar contains 5–8% acetic acid; see Experimental Section for additional manufacturing details). The original optical molds have rounded gratings with heights of $183.3 \pm 5.7$, $330 \pm 10$, and $150 \pm 5$ nm for samples with 300, 600, and 1000 lines $mm^{-1}$, respectively. While the grating periods are conserved in the chitinous replicas, their heights are significantly lower than those of the molds due to partial filling and vertical shrinkage during solvent evaporation,[27] resulting in chitinous grating heights of $66.67 \pm 5.7$, $96.67 \pm 5.7$, and $76.67 \pm 5.7$ nm, respectively. All the chitinous samples produce some iridescence, with intensities directly correlated with their periodicities, confirming previous observations in beetles.[20] Interestingly, the low heights and aspect ratios—the latter ranging from 1:0.06 to 1:0.15—of the chitinous structures debunk the commonly held assumption, arising from observations of iridescent and non-iridescent beetles, that structures with high aspect ratios are required to produce this effect[20] by demonstrating that topographies of tens of nanometers are sufficient.

## 3. Chitinous Color Characterization and Optimization

The color patterns observed in our chitinous gratings resemble those commonly observed in the transparent wings of many insects and butterflies.[28] In both contexts, the color is due to the constructive interference of light reflected from the thin chitinous layer, resulting in wings that are mostly transparent to the human eye but colorful for arthropods with more specialized vision extending into the ultraviolet and near-infrared regions.[28] A common strategy found in natural systems to enhance the intensity of reflected over transmitted light and to improve color saturation is the addition of a dark background to minimize incoherent scattering.[21] In insects, this effect is commonly achieved—in multiple ways—by using melanin, such as in black scales or a black basal lamina under the color-producing scales in the case of butterflies or in melanin-rich layers in the lamellae of beetles' elytra.[11–13,29] Interestingly, beetles living in concealed spaces also use melanin extensively—in their case, to achieve their dark color.[30] In our case, since the iridescence intensity is proportional to the grating pitch, we focus on optimizing the optical effect of the highest frequency grating (i.e., 1000 lines $mm^{-1}$) by covering the back (i.e., flat) surface of the freestanding chitinous gratings with a coating based on carbon black (**Figure 2**a and S2, Supporting Information).

Adding the black background to the chitinous gratings enhances the color purity, but with an unpredicted homogenization of the color along the surface (Video S1, Supporting Information). At a fixed angle, the uncoated samples show a diminished reverse-order spectrum (i.e., red followed by green and blue), transforming into an intense and largely homogenous color when back-coated (e.g., blue in Figure 2a). The transition from a largely uncontrollable iridescence, represented by a rainbow pattern, to an angle-controlled iridescent color, similar to that of beetles with multilayer reflectors, enables the mimicking of the biological functionalities of iridescent surfaces. For example, the combination of a color-homogenous chitinous pattern with a curvature (i.e., a geometry facing all incident angles simultaneously) enables the creation of an object that showcases the whole color palette simultaneously in a way similar to that is achieved by some iridescent beetles[13] (Figure 2b and Video S2, Supporting Information). Notably, this use of curvature to tune a surface's iridescence is not exclusive to beetles; peacock spiders, for example, use the same effect in reverse, with structures that produce a single intense color when curved but a combination of reverse colors when flat.[31] Curvature is also used in feathers to control iridescence.[22,32]

In our study, iridescence is measured quantitatively by collecting angle-resolved spectra from samples rotated with respect to a light source (Figure 2c), demonstrating a linear transition to different colors with a wavelength shift of about 17 nm per degree of rotation. This is qualitatively observed as a transition from blue to red via the whole color palette when the samples are tilted at 15°

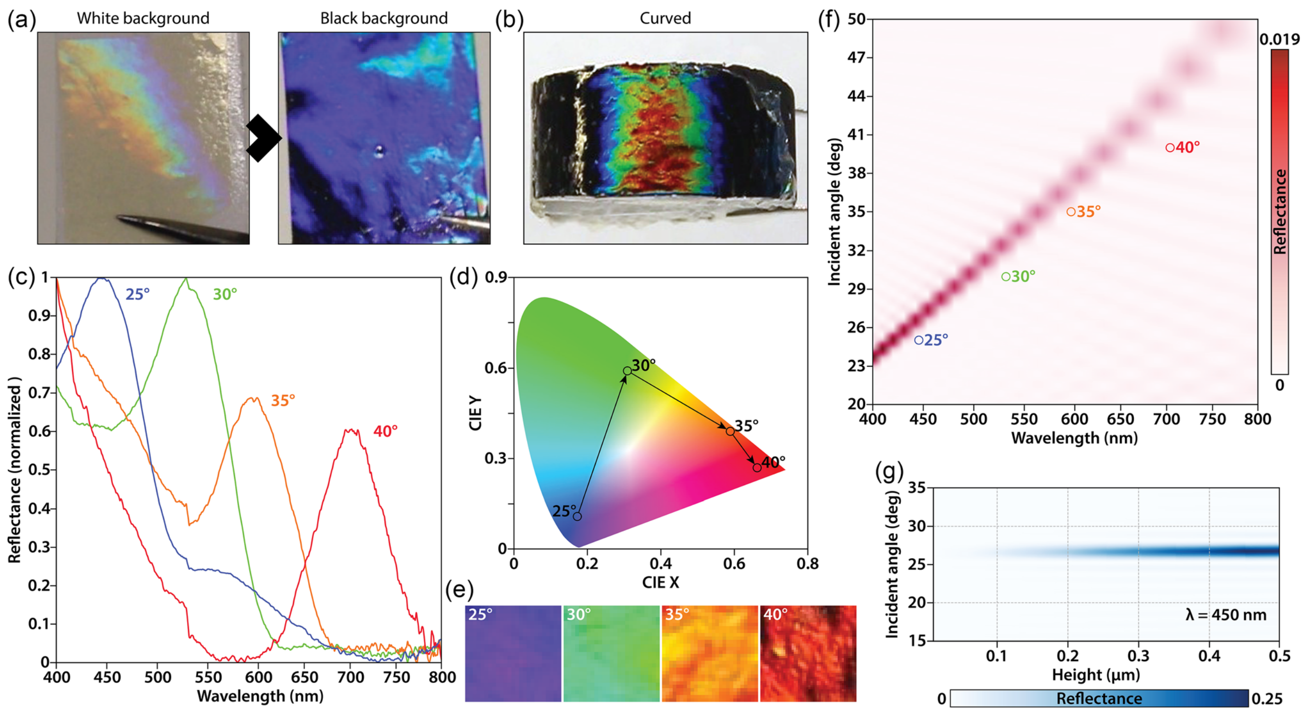


**Figure 2.** Optical characterization of chitinous films. a) Comparison of a 1.5 × 2 cm chitosan film with a 1 µm pitch grating both without (left) and with (right) a black background coating. The combination of a grating with a melanin-like background eliminated the rainbow effect (i.e., all colors present at all angles), establishing a fixed relationship between the angle of incident light and the color of the surface. See Video S1, Supporting Information, for further information on the iridescence effect of this sample. b) Image of the film with a black background and conforming to a curved surface. Combining an iridescent surface with a macroscopic curvature is a common strategy in natural systems to control structural coloration (see Video S2, Supporting Information). c) Characterization of the angle of incidence with the color of the surface. The chitinous surfaces show a linear relationship in which the wavelength of the reflected light shifts approximately 17 nm per degree of the observer–light source rotation. d) Displacement of the surface color in the CIE color space at different observation angles. e) Optical pictures of the chitinous gratings at different angles. f) Simulated far-field diffraction spectrum of the grating shown in (a) with a 1 µm pitch. The measured peaks from (c) are plotted as colored circles. Color intensity is proportional to reflectance (see Figure S3, Supporting Information, for further details). g) Simulated impact of the grating height on the diffraction intensities for blue wavelengths ($\lambda = 450$ nm) for the grating shown in (a). All optical images in this figure were taken under standard tungsten laboratory light (3200 K).

(Figure 2d,e). The angle-dependent reflectance arises because of the diffraction of light incident to the regularly spaced parallel ridges or grooves,[33] as verified by finite-difference time-domain (FDTD) electromagnetic simulations (Figure 2f and S3, Supporting Information). The grating structure splits the incident white light into component wavelengths that are then reflected at particular angles with varying efficiencies—the shorter wavelengths at lower incident angles are brighter and more saturated than the longer wavelengths at higher angles (Figure 2f).[17]

We also simulated the impact of the grating profile and density (Figure S4, Supporting Information). The grating profiles (i.e., semicircular/sinusoidal vs squared) have minimal impact on the simulated spectra. We were able to reproduce the qualitatively inferior iridescence observed in our samples with larger grating periods (i.e., 300 and 600 lines $mm^{-1}$) in the simulation, as seen by up to an order of magnitude decrease in theoretical diffraction efficiencies (relative to the 1000 lines $mm^{-1}$ grating). For the 1000 lines $mm^{-1}$ grating, only the first-order diffraction contributes to the light emerging from the sample at various angles. This corroborates the experimental observations in our samples and the assumption from the study of naturally occurring iridescent topographies in beetles, that structures with periodicities of 1 μm or less are required to produce this effect.[20] At small angles (less than 2°), the effect of the zeroth-order diffraction (i.e., the undiffracted light undergoing specular reflection) is dominant, even at 1000 lines $mm^{-1}$ (Figure S3, Supporting Information). This explains the enhanced saturation effect achieved by adding a broadband absorptive (i.e., melanin-like black) background that effectively attenuates the undiffracted light (Figure 2a and S2, Supporting Information).

A critical aspect of this research is the artificial production of chitinous structural color with characteristics achieved by natural systems—specifically, vibrant metallic iridescence using a version of beetles' chitinous gratings that have been optimized for artificial manufacturing. Removing the specular reflections using a melanin-like background is consistent with this aim and is easily implemented at a large scale. However, dark backgrounds are not the only strategy to enhance iridescence. Considering the simulation results, the chitinous gratings are still far from ideal from an optical standpoint because structures with a larger aspect ratio—closer to 1:1—would produce more intense iridescence (Figure 2g) that might overcome specular reflection without the need for an absorbent background. This, however, would be at the cost of scalability. In contrast, robust structures with low aspect ratios and absorbent backgrounds can produce chitinous iridescence similar to the best examples in beetles while being straightforwardly scalable. The success of the chosen approach becomes apparent when the patterned chitosan films are exposed to sunlight (instead of laboratory light) and compared to specimens whose iridescence is based on multilayer reflectors (**Figure 3**a and S5, Supporting Information). The implications are cross-disciplinary: levels of iridescence well beyond those shown in beetles can be achieved using biomimetic chitinous diffraction gratings, supporting the hypothesis that grating-based iridescence in beetles is a byproduct of another function, and there is now an alternative, using a fully ecologically integrated material, to conventional distributed Bragg or multilayer reflectors for artificially producing intense iridescence. A multilayer chitinous nanostructure, such as that in many beetles, would require alternating layers of materials with low and high refractive indexes, but fabricating such a structure would involve intense control of a chitinous polymer, a compatible material with a high refractive index, and customized tooling that does not currently exist. In contrast, optimized optical gratings created by casting commercial optical surfaces using hot roller embossing (i.e., with unrestricted size)[34] can achieve similar results and offer a new and straightforward method of producing robust structural color with a single layer, thus enabling a scale of manufacturing that is well beyond that of laboratory samples and can incorporate chitinous iridescence in large-scale products.

## 4. Large-Scale Production of Chitinous Color

There are immediate practical applications for our new method. Optical features, such as color, are a central aspect of product design,[35] providing both information and appeal, but in the case of plastic consumables, such colors clash with the goal of recovering and reusing polymers because existing covalently bonded pigments require an additional sorting step that is impractical for conventional waste management systems.[36] With the ability to produce chitinous color with a single-layer topography, we can apply commercial holographic diffraction gratings produced by hot embossing to cast chitinous gratings measuring tens of centimeters, enhancing and saturating with iridescent color when combined with a dark background (Figure 3b).

This represents a qualitative step forward in the artificial production of structural color with chitinous polymers.[6] More importantly, previous attempts to produce sustainable color using a biodegradable material have focused on modifying naturally occurring molecules (e.g., cellulose) to achieve a material that is manufacturable using conventional tools.[37,38] However, due to the stability of structural biomolecules, such modifications require highly bioactive chemicals (e.g., propylene oxide to form hydroxypropyl cellulose or chloroacetic acid to form nanocellulose), with health and environmental impacts that greatly exceed the benefits of using a biodegradable component.[39,40] The biomimetic manufacturing we employ is the first artificial method for reproducing a natural structural color using native components and avoiding chemical modifications, with the added significance that the material used is the second most abundant organic molecule on Earth and responsible for some of the most sophisticated optical structures and functionalities in nature (e.g., butterfly, beetle, and crustacean cuticles).[11–13,17] The large scale of the color created in this study, particularly considering the fundamental aspects of the material used, enables the first fully ecologically integrated structural color for large objects and products. Therefore, this study significantly contributes to the field by successfully reproducing the chitinous structural color of the arthropod cuticle in a controlled manner. This is a marked departure from the production of cellulosic gratings, which are not relevant to this field. In terms of sustainability, conventional cellulose work necessitates using strong organic solvents to break down robust intermolecular bonds. Therefore, despite its abundance and biodegradability,

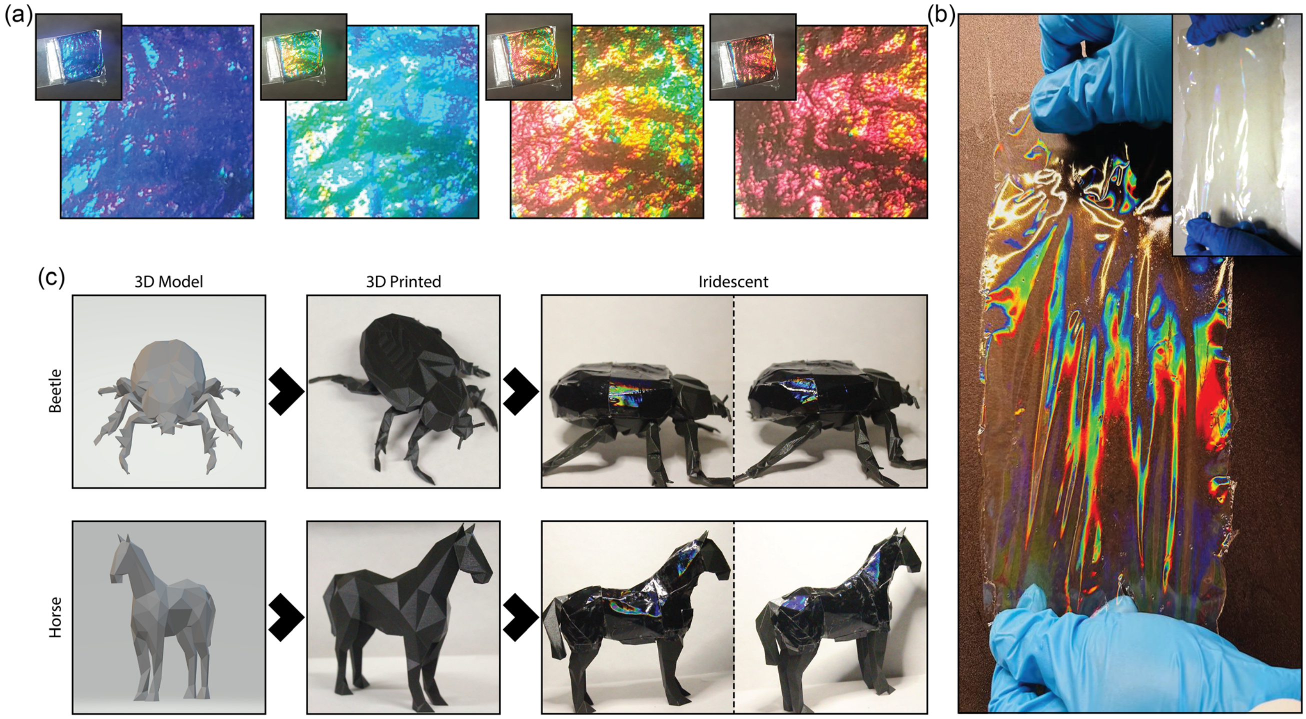


**Figure 3.** Large-scale production of chitinous iridescence. a) Examples of a 1 μm pitch grating made out of chitosan with a black background under sunlight. The inset of the image shows the picture of a 2.5 × 2.5 cm sample mounted on a glass slide as it is rotated with respect to the camera. The larger image is a close-up picture of the actual sample. Refer to Figure S5, Supporting Information, for a comparison with metallic coloration based on the multilayer reflectors of dead-nettle leaf beetle (*Chrysolina fastuosa*). b) Image of a 13 × 22 cm large iridescent film of chitosan with a 1 μm pitch grating. While this sample is uncoated, the role of the background in enhancing the iridescent effect can be qualitatively observed when the sample is placed in front of a black (large image) or white (inset) background. These films represent an advance in scale of sixteen orders of magnitude toward the production of fully biodegradable chitinous structural color. See Video S2, Supporting Information, for further information. c) Examples of black polylactic acid 3D-printed models of a beetle (15 cm long) and a horse (18 cm tall) to which the films in the previous panel have been applied to form a conformal iridescent layer. The rightmost panels are images of the two objects at different angles. See Video S3, Supporting Information, for more details on the models.

the applications of cellulosic materials are limited due to the environmental impact of these solvents. Contrastingly, our chitosan treatment process employs acetic acid at a concentration of 1%, which is within the anaerobic limit. This approach presents a realistic and sustainable alternative that can be seamlessly integrated into highly efficient circular manufacturing models.[23]

To illustrate the possibilities, we present two simple models of organic shapes (a beetle and a horse; see Figure 3c) modified to have flat surfaces at different angles. The models are printed using dark green polylactic acid (PLA), and large structured chitinous films are attached to the surface, creating a conformal coating. The objects show the strong iridescence of the chitinous films on different surfaces as they change positions with respect to the light source and camera (Video S3, Supporting Information). Notably, while the PLA core is not biodegradable under natural conditions, a biodegradable coating without additives or pigments allows color to be applied that can later be selectively recovered or metabolized, after which the core of the object can be processed in single-stream recycling (i.e., without color sorting). When added to the ability of chitosan to produce consumables,[41] our demonstration of large-scale chitinous structural color points the way to the production of biodegradable chitinous objects with fully integrated color but without the need for recovery, sorting, or any other human intervention in the surrounding ecosystem.[23]

In conclusion, we used bioinspired chitinous manufacturing to shed light on the iridescence of certain beetles and applied those findings to fabricate the largest samples of chitinous structural color to date. Microscale control of the chitinous structures enabled the production of replica of coleopteran cuticles with characteristics tailored to a context largely unexplored by evolution: the optimization for iridescence of chitinous gratings in the cuticles of beetles that live in concealed areas. We showed that optimizing these structures using materials and principles native to the beetles produces iridescent colors via diffraction that are comparable to the iridescence of the multilayer reflectors of leaf beetles. Our work offers an alternative and easily scalable route to multilayered reflectors for producing vibrant and iridescent chitinous colors based on a grating topography and a dark coating, which we combined with 3D-printed geometries to produce large objects with biodegradable chitin-based iridescence, thus pointing the way to incorporating structural color into purely chitinous products.

 

Notably, the approach used in this work stands in contrast to the common approach to bioinspired engineering. That approach aims not to reproduce the best solution—achieved under the rules and constraints of evolution—from biological systems for a given problem, but to force a kind of "artificial targeted evolution" by optimizing a largely unevolved solution with potential for engineering adaptation. We believe that the direction we have taken will inspire greater consideration of the possibilities nature has already realized over millions of years of optimization—especially of "spandrels" or features that are byproducts of selection for some other function.[42]

## Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

A.K. and J.G.F.: conceived the idea of creating large-scale chitinous color inspired by the Coleoptera cuticle. A.K.: produced the physical samples and their characterization. C.F. and V.S.: provided expertise on the biological aspects of arthropod color. J.G.F.: selected and characterized the biological specimens. V.S.: performed the FDTD simulations. J.G.F.: designed the 3D-printed objects, which AK printed. J.G.F. and A.K.: wrote the first draft of the article. All authors contributed to the editing of the final manuscript.

## Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

## Keywords

chitin, Coleoptera, cuticle, iridescence, structural color

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