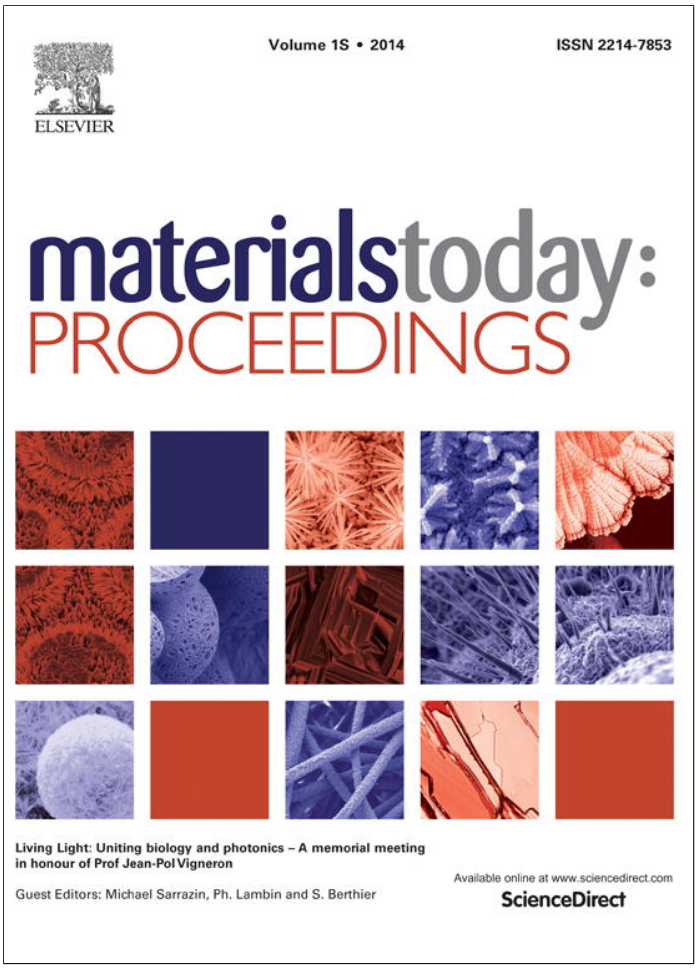

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Living Light: Uniting biology and photonics – A memorial meeting in honour of Prof Jean-Pol Vigneron

Bouligand Structures Underlie Circularly Polarized Iridescence of Scarab Beetles: A Closer View

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Abstract

The iridescent metallic green beetle, *Chrysina gloriosa*, selectively reflects left circularly polarized light, and exhibits iridescence. The exoskeleton of *C. gloriosa* is decorated by a polygonal texture: hexagonal cells (~10 micron) coexist with pentagons and heptagons. We find that the fraction of pentagons and heptagons computed using Voronoi analysis increase with an increase in curvature, implying that it is energetically favorable to create disordered patterns for higher curvature surfaces. In bright field microscopy, each cell contains a bright yellow core, placed in a greenish cell with yellowish border, but the size of the core and spatial distribution of color changes when the angle of incoming incident light is varied. Using confocal microscopy, we observe that these cells consist of nearly concentric, nested arcs that lie on surface of a shallow cone. We infer that the patterns are structurally and optically analogous to the focal conic domains formed spontaneously on the free surface of a cholesteric liquid crystal. In this paper, we describe in detail how the microstructure referred to as Bouligand structure provides the basis for the morphogenesis, and for generating the intricate optical response of the exoskeleton of the beetle.

Selection and Peer-review under responsibility of Physics Department, University of Namur.

Keywords: Cholesteric liquid crystals, structural color, iridescence, jewel beetles, Chrysina gloriosa, Vornoi analysis, focal conics, Bouligand.

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1. Introduction

Colors of many plants and animals are not based on pigment and dyes (biochromes); in fact, some of the brightest and most fascinating colors result from the interaction of light with structures on the micro- and nano-scale [1-8]. The structural origin of color of various butterflies [1, 2, 6, 8, 9], birds [10-12], moths [13] and plants [14, 15] is often identified with thin film interference, multilayer interference, diffraction and scattering [1-5, 13, 16-20] and the color may have contribution from the pigmentation in the underlying layers. Furthermore, colors of many insects and birds change with visualization angle, exhibiting iridescence [1, 2, 19, 21], quite like the interference colors seen in thin soap films or bubbles [22]. The term "iridescence" used in different fields of study can confer on it different characteristics; therefore, in this article we use the term iridescence to mean "a change in the hue of the object possessing the perceived color as the angle of vision is varied", a definition quite similar to what Mason used in 1927 [23]. In the language of color science [1, 24], perception of isolated color (see discussion in ref. 1) can be identified and specified using three quantities: hue (for example, how yellow differs from red), brightness (qualitatively associated with sensation of overall intensity, ranging from dim to dazzling) and saturation or purity of color. The comparison of iridescent colors in butterflies with thin film colors is based on the following clues [1, 17, 25]: absence of dyes or pigments, shift of color (or reflection maximum) to shorter wavelengths with increasing angle of incidence and the change of color towards the red end of spectrum when wings are immersed in fluids that have higher refractive index than air. For a very large number of birds, butterflies, and animals as well as some plants, the color produced by the organism and perceived by the observer is a result of interaction of light with the periodic structures on their bodies, and iridescence shows trends consistent with this behavior [1, 5, 25]. However, even though certain beetles exhibit structural color, in contrast with most birds and butterflies, there is a twist to their reflectance [25-28]: the reflected light is circularly polarized (typically with left handedness) and a red shift is observed on increasing angle of incidence (or for oblique incidence). The present article examines with the structural origin of iridescence (with the twist) exhibited by scarab beetle *Chrysina gloriosa* or *Plusiotis gloriosa.* The scarab beetle *C. gloriosa* possesses a metallic green reflection that is circularly polarized [29-32].

The natural world is full of color stimuli, and humans and animals have evolved their complex visual systems to identify, distinguish and respond to colors in nature [33-38]. Interestingly, while human eye can cope only with hue, brightness and saturation, many animals have evolved to perceive and use the natural and artificial polarization [35, 36, 38]. Electromagnetic radiation has electric and magnetic fields that are perpendicular to the direction of propagation. In the case of so-called randomly polarized light, the electric field vector oscillates in a random fashion. Polarized light comes in two flavors: linearly polarized light has electric field oscillating in a single direction while the field can rotate at the optical frequency for circularly (or elliptical) polarized light. Scattered and reflected light from the sky is predominantly linearly polarized, as is underwater light. Likewise, light reflected from polarizing layered structures found on various species of insects, birds, fishes and plants is typically linearly polarized [35]. Though linearly polarized light is quite commonplace in nature, the selective reflection of circularly polarized light is somewhat rarer [35, 39].

The selective reflection of circularly polarized light by beetles was first observed by Michelson [40]. Nearly fifty years later, in a series of papers Robinson [41], Bouligand [42, 43] and Neville & coworkers [44, 45] provided critical analysis and experimental evidence to show that the optical properties as well as "ultrastructure" revealed in electron microscopy of beetle cuticle are quite similar to cholesteric liquid crystals. Cholesteric liquid crystals (CLCs) possess long range orientational order, described by a unit vector **n**, known as the director, and their equilibrium director structure is a helix [46, 47]. The director advances uniformly tracing a helix of pitch p. The cholesteric phase due to its helical structure exhibits selective reflection when the pitch of the helix is comparable to the wavelength of visible light, peaked at a wavelength λ_0, given by $\lambda_0 = np$, where n is the average refractive index [48]. The spectral width of the reflection peak for a pure cholesteric phase is related to birefringence ($\Delta n = n_e - n_o$) by $\Delta\lambda = p\Delta n$ where n_o and n_e are the refractive indices for polarizations perpendicular (ordinary) and parallel (extraordinary) to the axis of anisotropy, respectively. The reflection has the same handedness of the cholesteric helix. Bouligand [43, 49-51], who carried out very extensive studies on cholesteric liquid crystals (CLCs), provided detailed three-dimensional models of textures and morphology of CLCs, and contrasted these with textures observed in crabs and other organisms. He argued that the formation of CLCs has role in

morphogenesis [42, 49-53]. Inspired by Bouligand's contributions in this context, Pace described the helicoidal structure found in beetle *C. gloriosa* as 'Bouligand structure' [31].

Most recently, we examined the morphology and optics of the scarab beetle, *C. gloriosa* and showed for the first time [29] that (a) the cellular polygonal pattern exhibits curvature dependant disorder, (b) the complex architecture of the beetle is structurally very similar to focal conic domains formed by cholesteric liquid crystals near a free surface, in particular to structures reported by Meister et al [54, 55] (use of non-evasive confocal microscopy relying on auto-fluorescence of an unidentified fluorophore was another remarkable feature of the study), and (c) within the polygonal cells, the visualized colors depend critically upon the angle of incidence. The beetle, the polygonal texture at the top imaged using optical microcopy and confocal microscopy are shown respectively in Figure 1. On examining transmission electron micrographs from the exoskeleton of *C. gloriosa,* Pace [31] also noticed a polygonal texture on the top surface. Even though the cuticle exists as a solid composite, so different in appearance and properties from fluid-like cholesteric liquid crystals, the texture and optical properties are remarkably similar. The analogy allows us to at least qualitatively explain the iridescence and the so-called red shift observed when the angle of incidence is made increasingly oblique. Since the arguments supporting structural origin of circularly polarized iridescence were presented earlier with brevity appropriate to a report [29], we reiterate and detail those contextual arguments here. We provide a historical context that has culminated in the current understanding, and provide critical additional evidence that links the exoskeleton morphology to its artificial analogues and to morphology observed in other organisms.

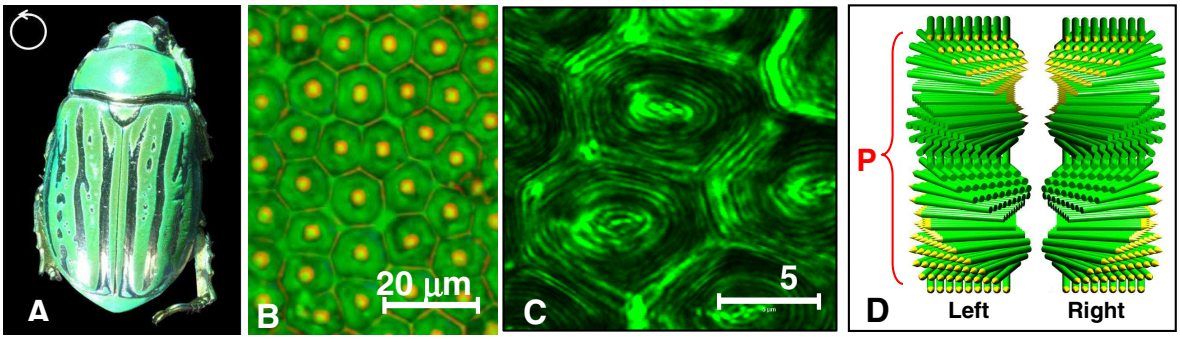

Fig. 1: (A). Photograph of the beetle *C. gloriosa* displaying the bright green color with silver stripes as seen in unpolarized or with left circularly polarized light; (B) An image using a reflected light microscope of the exoskeleton of the beetle *C. gloriosa* showing bright yellow core surrounded by greenish reflection; (c) An x-y section using a confocal light microscope, showing the concentric rings resolved at high magnification and present near the free surface; (D) A schematic representation of the cholesteric helix for both handedness.

2. Historical Background

The connection between structures and the iridescent color was made early on by Hooke [56]. He observed that the brilliant colors of peacock and duck feathers were destroyed by a drop of water, and postulated that alternating layers of thin plates and air might strongly reflect the light. Newton[57] understood that the colors produced in bird feathers including peacocks must be due to the presence of "thin film structures". Many researchers like Lord Rayleigh (the son) [28, 58], Mason[23], Onslow[25] and Land[17] cited experimental evidence to suggest that colors of many insects and organisms are created by physical mechanisms that do not rely on absorption or emission of light by biochromes or pigments. Even the early researchers recognized the optical effects observed in beetles were at variance with the observations for butterflies and birds (e.g. see discussions in Onslow paper [25]).

Scarab beetles are often called "jewel beetles" as they are used as ornaments in many Asian countries [59]. Michelson [60, 61] (1911) was first to note that some scarab beetles possessed a metallic reflection and that the reflection was circularly polarized. Specifically, Michelson [60] studied the color of beetle *Plustiotis resplendens* and noted: "This is a beetle whose whole covering appears as if coated with an electrolytic deposit of metal, with a lusture resembling brass. Indeed, it would be difficult for even an experienced observer to distinguish between the metal and the specimen". While *C. gloriosa* is green in color, it does possess the metallic luster that Michelson refers to. In his study, Michelson[60] noted that the reflection was circularly polarized but did not specify the handedness, although he did mention that handedness reverses if one were to look at the reflectance from the blue

part of the visible spectrum to the red – the polarization was found to reverse near the red end of the spectrum and was completely reversed in the extreme red. Furthermore, though Michelson correctly postulated that "the effect must therefore be due to a "screw structure" of ultra microscopic, probably of molecular dimension", he did not pursue this further. Several researchers including Gaubert (1924) [62] and Mathieu & Faraggi [63] (1937) compared the optical properties of beetles to liquid crystals formed by cholesteryl derivatives.

By the time, Robinson[41] took up his studies on beetles in 1966, understanding of textures, patterns, thermodynamics and optics of liquid crystals had expanded considerably, (due to efforts by Friedel [64], de Vries [65] and other pioneers [66]), and he extended the comparison of optical properties between beetles and cholesteric liquid crystals by including CLCs formed by synthetic polypeptide dispersions [67]. Later Bouligand [42, 43] as well as Neville and co-workers [44, 45] pursued the origin of circularly polarized reflection using electron microscopy on several scarab cuticles and found that even the architecture is analogous to structures postulated in cholesteric liquid crystals [64]. Thus, Bouligand and Neville laid the basis for the understanding that "helicoidal structures" are responsible for the color (selective reflection) and the handedness of the circular polarization. More recently, Goldstein [30] and Arwin et al [68] summarized the history of optical measurements made in scarab beetles and performed ellipsometric studies confirming their polarizing behavior.

On observing polygonal structures in the exoskeleton of *C. gloriosa*, we reasoned that these patterns could be a result of a characteristic texture observed in cholesteric liquid crystal literature. In typical textbooks, the polygonal structure of cholesteric focal conics is drawn based on Bouligand's classic paper from 1972 (see texts by: de Gennes and Proust [46]; Oswald and Pieranski [47]; or Kleman and Lavrentovich [69]), and on a cursory glance, it looks different from imperfect hexagonal arrays present on the beetles. But when we stumbled upon the detailed study of textures formed in a siloxane based oligomer carrying two mesogens published by Meister et al [54, 55], the similarity between the textures reported by them to those observed in *C. gloriosa* was unmistakable [29]. In synthetic samples of Meister et al [54, 55], the cholesteric texture formed can be preserved by quenching the samples below glass transition.

The cuticle of many beetles (and crustaceans) is composed of chitin-protein complex [45]. Many polysaccharides form rigid-rod like nanocrystals or microfibrils through interchain hydrogen bonding [70-75], and their nanocrystal suspensions exhibit a cholesteric liquid crystalline phase [74]. During the formation of the cuticle of beetles, over hundred proteins and other biomolecules interact with chitin (a polysaccharide based on N-acetyl-β-D-glucosamine [76]) to produce microfibrils that self-organize to form cholesteric liquid crystals, eventually resulting in observed morphology and in optics with a twist [45, 77-79]. The circular polarizing reflectors appear to be limited to a narrow group of beetles, namely the Scarabaeidae family and further to the subfamilies such as Rutelinae, Scarabaeidae and Cetoniinae[27]. Leaving aside the obvious, but poorly understood questions about biological function of color (discussed later), we focus our attention to patterns observed in a scarab beetle called *C. gloriosa*, with the aim of understanding its complex architecture and colors. Here, we describe how the origin of metallic sheen and selective reflection of circularly polarized iridescence of this beetle lies in the interaction of light with the patterns and microstructure formed by the cholesteric liquid crystal embedded in its exoskeleton.

3. Results and Discussion

In randomly polarized daylight, the scarab beetle *C. gloriosa* selectively reflects left-circularly polarized light and possesses a brilliant metallic green appearance (see Fig. 1A). If left circularly polarized light is blocked by the use of a right circular polarizer, the beetle 'loses' its characteristic bright green reflection [29-32]. The reflectance of *C. gloriosa* beetle has a broad halo from 500-600 nm with two peaks at 530 (green) and 580 nm (yellow), respectively. The body of the beetle when observed under a reflected light microscope consists of a richly decorated mosaic of regularly spaced polygons that cover the entire cuticle of the beetle. When viewed in brightfield microscopy (see Fig. 1B), the structure seems to consist of mostly hexagonal cells (~ 8-10 μm), and each cell has a bright yellow core surrounded by green. Similar cellular pattern has been observed for another jewel beetle called *P. boucardi [80]*. To better understand the structure of the beetle exocuticle, we used a laser scanning confocal microscopy (Leica TCS-SP) to reconstruct a three-dimensional map of the underlying structure using the auto-fluorescence of the beetle. The fluorescence was excited by the 488nm laser line of an Argon ion laser. The relief of the cells, when observed under high magnification, reveals itself to consist of bright and dark, nearly concentric regions (Fig. 1C).

The auto-fluorescence response from the unidentified fluorophore present in the elytra is dependent on the polarization state and intensity of incoming light. Hence the darker regions could point to either changing

fluorophore orientation or to a change in the fluorophore concentration [81, 82]. The 3-D reconstruction of the images suggests the former and it shows the existence of a microstructure of nested arcs and the cells seem to have a conical protrusion at the center (Figure 2). The cells under the fluorescence microscopy exhibit nearly concentric bands that are bright and dark which we attribute to the underlying structure of the exocuticle: the 3-D reconstruction suggests the existence of nested arcs. In our previous report[29], we had concluded that the structure that is found by confocal microscopy on the beetle exocuticle is completely analogous to the structure found on the free surface of a synthetic cholesteric liquid crystal reported by Meister et al [54, 55] using AFM studies. A detailed and careful survey of patterns reported for cholesteric liquid crystals reveals that the concentric circles apparent in confocal microscope image (see Fig. 1C) are also observed in polypeptide dispersions reported by Robinson [41], chitin and cellulose dispersions [70] as well as in certain conjugated polymers [83]. Historically scientists studying liquid crystals as well as beetles have argued that it is not possible to cut these samples in a way, which does not perturb the structure [54, 55, 84, 85]. According to Bouligand [84, 85], the surfaces produced by the freeze fracture process for investigations with electron microscopy can possess a relief due to the anisotropic propagation of fracture and due to the action of a microtome knife. As we imaged the three-dimensional microstructure of the beetle elytra in a non-destructive fashion using confocal microscopy, the question of microtome-related artefacts does not arise.

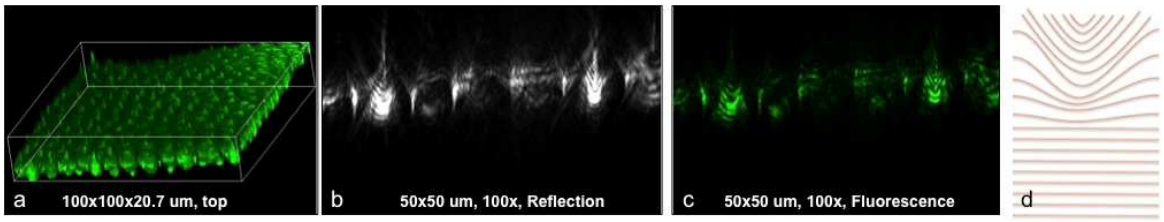

Fig. 2: Fluorescence confocal microscopy images of the cellular exoskeleton of the beetle *Chrysina gloriosa*, obtained using excitation at 488nm. 3D reconstruction of the beetle microstructure is shown in (a). Side views (xz section) of the skeleton obtained in reflection mode (b) and in fluorescence mode (c). The seemingly concentric bright/dark regions in Figure 1c are actually due to the multiple layers of bright/dark regions in the shape of nest arcs, which eventually turn into a conical frame at the top. The apparently incomplete cones/spikes are located behind the complete ones, hence not fully in focal plane resulting in such images. (d) Schematic of the microstructure of the nested arcs. The shallow conical frame at the top is not sketched.

As the cellular organization on beetle exoskeleton appears to be hexagonally ordered, we decided to characterize the extent of hexagonal order in patterns using Voronoi analysis, a method typically used in materials science for patterns recognition and for modeling the properties of spatial structures [86] as well as studying phase transitions and order in colloidal assemblies [87-89]. The Voronoi cell (or Dirichlet region) is the smallest convex polygon surrounding a point, whose sides are perpendicular bisectors of lines between the point and its neighbors [86]. We imaged different parts of the beetle with a reflectance microscope and mapped the centroids of cells using Image pro. These datasets were used to construct Voronoi polygons using Matlab codes, as illustrated with an example in Figure 3. The regular lattices of cells contain not only hexagonal cells but also cells that are pentagonal and heptagonal. It was also noticed that, as the curvature of the beetle exocuticle increased, the number of heptagons decreased a little and that of hexagons decreased more, while that of pentagons increased most [29]. In other words, the more curved the surface of the beetle was, the more number of pentagons was found, thus leading higher disorder with increasing curvature. While hexagonal packing affords the most efficient use of space on a plane, defects (pentagons and heptagons) are essential for tessellating a curved surface, thus leading to higher disorder of the structures on the head, thorax and the abdomen of the beetle due to the curved nature of the body of the beetle. Research efforts in spherical crystallography [90-92] illustrate the importance of grain boundaries and defects in creating minimum-energy configurations for the curved substrates. According to Nelson [91], the energetic cost associated with creating defects scales as YR^2, where Y is the two-dimensional Young's modulus and R is the curvature radius. Since the cost becomes substantial for systems with large R/a, (a is characteristic length: e.g. the size of particles in a colloidosome) the system reduces this energy in one of two ways: A buckling transition can occur for hollow spherical shells, thus rationalizing the faceted morphology of viruses [91]. Conversely, if surface tension limits the buckling out of the local tangent plane, disclinations can emanate grain boundary scars as have been observed for particles packed on a spherical droplet, (a colloidosome) [90]. Thus in

the context of *C. gloriosa*, the disordered hexagonal morphology (Figure 3) as well as shallow cone at the center of every cell (Figure 2) are possibly a result of similar energy minimization.

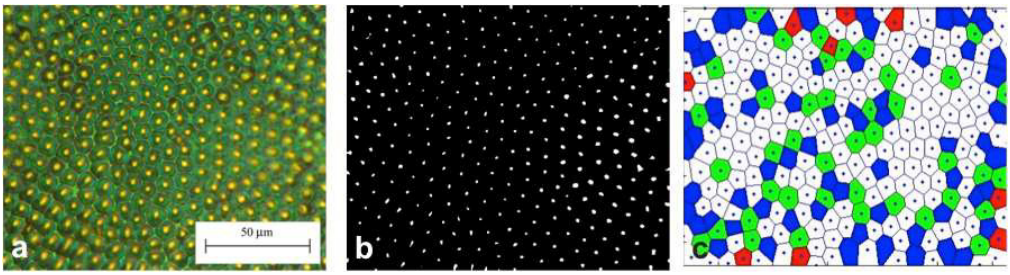

Fig. 3 a) Original image of cellular pattern obtained using the microscope. b) Image mask exposing the centroids. c) Voronoi analysis of a section from the corresponding image. Pentagons are colored blue, heptagons are red and hexagons are white. The fraction of pentagons and local disorder increase with curvature.

We have argued that the formation of cellular pattern, organization of chitin within the cuticle as well as the shallow cone observed using confocal microscopy on this beetle are quite analogous to structural features reported by Miester et al [54, 55]. In fact, Miester et al [54, 55] argued that the interplay between surface tension and the anchoring and distortion energies results in the microstructure of the focal conic domains formed from a cyclic siloxan oligomer bearing two mesogens, and visualized using atomic force microscopy and electron microscopy. In presence of free surface where interface exists between a liquid crystal and isotropic phase, the surface reliefs can appear. The creation of patterns, defects and disclinations at the free surface belong to a class of problems known as free-boundary variational problems. Qualitatively, we can infer that the pattern observed on beetle exoskeleton is a result of the influence of the anchoring conditions at free surface, in addition to the physiochemical properties of chitin-protein suspension that self-organizes into cholesteric phase. Using suspensions made with chitin extracted from purified shrimp and crab, Revol, Marchessault and coworkers [74] showed that characteristic chiral ordering of cholesteric liquid crystals can be realized *in vitro,* and both helicoidal morphology and fingerprint-like pattern are manifested. Similar observations have been reported for textures created from other polysaccharides [70, 72-75]. Furthermore, the order is preserved in dried samples, though the periodicity (and pitch) changes on drying. Thus the imperfect hexagonal array is indeed a focal conic texture produced by cholesteric liquid crystals, when this equilibrium structure results from a competition between surface and bulk energy.

Having attributed the polygonal structure on the beetles to an array of focal conic defects, we next provide an explanation for the iridescent colors. When unpolarized light is incident on the cholesteric helix, with the helical axis oriented normal to the surface, it reflects 100% of the light with the same handedness. Unpolarized light can be thought of as a mixture of left circularly polarized and right circularly polarized light, and therefore, light of the same handedness (~ 50%) is reflected while the rest is transmitted. It should be pointed out that there is very little absorption in a cholesteric fluid; however, that may not be the case for the beetle exocuticle. In focal conic textures present in the beetle described here, light incident on the beetle interacts with nested arcs where the orientation of helical structure is continuously changing. As was argued in our previous report [29], the optical properties of such a structure are quite complicated [48, 93], and require a description corresponding to oblique incidence where interaction of light with a perturbed focal conic texture embedded in a planar texture are considered. The red shift in wavelength on increasing angle of incidence can be shown in an optical microscope (see Fig. 4) by either varying aperture size in bright field mode or using dark field mode (where only obliquely incident rays are allowed). The red shift is simply a result of locally satisfying pseudo-Bragg reflection at lower angle (alternatively, for obliquely incident light on a helical structure that is not parallel to beetle surface, the local angle of incidence becomes smaller and smaller as apparent angle at air-material interface is increased). In a recent study Agez et al [93] used same oligomer as Meister et al[54, 55] and showed that longer annealing times lead to textures that are artificial mimics of the morphology seen in *C. gloriosa*. Furthermore, the authors also found color contrast and changes on angle of incidence consistent with our observations, and likewise they concluded that the spatial distribution of color observed is due to variation in helical axis orientation with respect to the air-material interface. Thus we surmise the patterns that are found on these beetles are largely a consequence of the array of focal conic defects and the color is due to the selective reflection mediated by the defect array on the surface of Bouligand structure. This explanation is ssomewhat different from that offered for color generation in other beetles *[80, 94-*

96]. For example, both *P. boucardi [80]* and the New Zealand manuka beetle [94], the presence of chiral reflectors with two different pitches was cited as responsible for color.

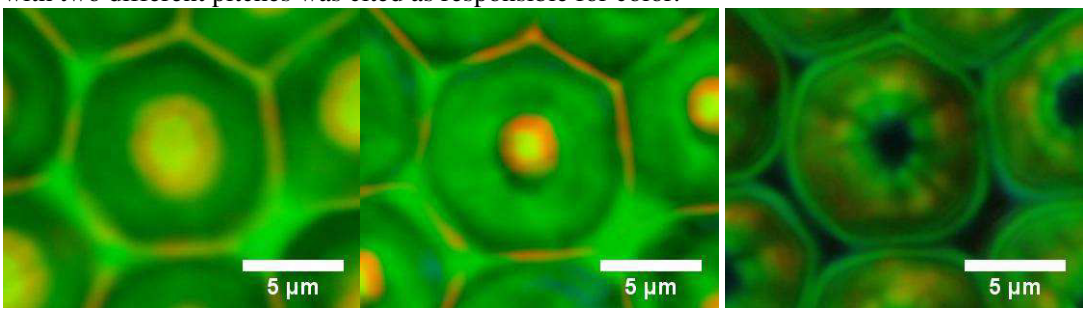

Fig. 4: Optical micrographs of *C. gloriosa* under microscope (A) bright field, open aperture or (B) bright field, closed aperture and (C) dark field. Increasing the aperture size in bright field broadens the range of incident angles, and near-normal incidence is absent in dark field illumination.

The perceived color of *C. gloriosa* is metallic green. We argue here that color mixing results in perception of single color, for interaction of light with microstructure underlying different regions within each polygonal cell produces a peak in intensity for very different colors (wavelengths) (see Fig. 4). There are a few other beetles and butterflies that use color mixing to produce iridescent colors [1, 5]. The beetle *Calidea panaethiopica* exhibits a complex color pattern that contains blue-green iridescent stripes. In this case the color is produced by a multilayer structure that has tiny cups with the cups producing two different colors that are again color mixed to provide the perception of a single color. Such is the case also with the butterfly *Papilio palinurus*; each wing scale is about 120 mm long with 5-10 µm diameter bowls, each lined with a multilayers stack of alternating layers of chitin and air [97, 98]. The distinct green color of the wing results from an additive color mixing of yellow and blue reflections. The yellow colored reflection is from the center/bottom of the bowl and blue results from two reflections at 45^0 at the edge of the bowl. It is remarkable that the natural world has a number of different but elegant solutions to producing iridescent colors for a variety of purposes.

The progress in understanding of defects and optics of cholesteric liquid crystals as well as biological analogues found in nature owes a huge debt to the elegant studies published by Yves Bouligand [42, 43, 49-53, 84, 85]. In fact, driven by the belief that Bouligand could have commented on the paper by Pace that identified cellular patterns observed in *C. gloriosa*, we looked meticulously through all the papers published by him and found a paragraph in a conference proceeding that corroborates our findings quite nicely [53]. (This paper was presented in V International Liquid Crystal Conference: it does not appear on ISI Web of science or google scholar or among papers citing Pace's article). In 1975, Bouligand [53] did not have the kind of evidence that we provide, yet in his characteristic style, he argues as to how and why morphogenesis can create polygonal fields generated on *C. gloriosa*. Since Bouligand Structures underlie scarab beetle iridescence, we dedicate this manuscript to his memory (see obituary [99]), and are convinced that the sheen of his work will continue to dazzle many scientists.

Insofar as the colors and circularly polarized reflection are created by an underlying cholesteric phase, one might wonder about the purpose of such colors and the resulting polarization. In the text "*Polarized Light in Animal Vision*" published in 2003, the authors reiterate this question by saying [35] "whether the eyes of these animals are able to detect circularly polarized light…The answer is unknown". As for the purpose of detecting circularly polarized light, they write, "the biological function, if any, of this phenomena is completely obscure." Questions like these are just beginning to be answered. Brady and Cummings [100] studied the response of *C. gloriosa* toward different light stimuli. They found that these beetles exhibit flight orientation that is dependent on the polarization of the light thus showing that these beetles in fact are sensitive to circular polarization of the light. It is possible that this sensitivity to circular polarization allows *C. gloriosa* to communicate in some fashion as the signals are independent of their orientation. However, another study published by Miklos et al[101] challenges these results, and concludes there is no evidence of circularly polarized light sensitivity in behavioral responses exhibited by several scarab beetles. Though the perception of circularly polarized iridescence continues to be an unresolved puzzle, it must be emphasized that the helicoidal structure formed provides the beetle with a material that has exceptional structural, mechanical, chemical and thermal properties [45, 102, 103]. It is possible that the primary objective of morphogenesis was creating body armor, but the formation of CLC textures spontaneously led to spectacular colors.

4. Summary and outlook

In brief, our report a) applies the Voronoi analysis to characterize the extent of order observed in optical micrographs of the imperfect hexagonal pattern found on the beetle exoskeleton, and infers that disorder increases with curvature (possibly to minimize the energetic cost of creating patterns on curved surfaces), b) uses non-evasive confocal microscopy, and auto-florescence of exoskeleton to characterize the microstructure and morphology of the exoskeleton of beetle, highlighting that these are similar to focal conic domains formed spontaneously on the free surface of a cholesteric liquid crystals and c) deduces the basis for circularly polarized iridescence of *C. gloriosa*, using measured optical response and analogy to optics of cholesteric liquid crystals.

There is a recent surge in interest in structural color, driven by the desire and need for multifunctional properties, often requiring high degree of control over reflectivity and additional properties like wetting [104-108]. Furthermore, the sensitivity to changes in periodicity or refractive index contrast brought out by sorption of vapors or solvents and say swelling-deswelling of microstructures responsible for structural color can be used for creating sensors that respond to chemical, thermal or mechanical stimuli [109-111]. Recent advances in optical characterization, synthesis and self-assembly, computer simulation as well as search for photonic and photovoltaic materials are driving advances in design and realization of desirable optical properties in biomimetic and bioinspired materials [106-108, 112-116]. The study of iridescent beetles shows there is rich complexity in structural colors found in nature [68, 100, 117-119], and there are many unsolved problems yet related to the specifics of how light interacts with micro and nanoscale patterns, including chiral structures as well as how these patterns are produced in nature [6, 94, 114-120].

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