Study of color variation in the *Phanaeus kirbyi* beetle at low temperature

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J. Flemming[1*], L. A. Feola[2] and A. S. Lavino[2]
[1] Physics Department, Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brazil
[2]Biology Department, Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brazil

E-mail: flemming@uepg.br

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Abstract

We analyzed the color change of the exoskeleton of the *Phanaeus kirbyi* beetle when it is cooled from room temperature to 5K. A blue shift of about 10nm was expected, due to the contraction of helical structures that would be responsible for the circular Bragg reflection and the consequent circularly polarized light observed in the insect. The expected shift to blue cannot be confirmed, as the RGB analysis showed non-spectral colors. However, a darkening of the exoskeleton with cooling can be observed and a didactic explanation of several possible physical causes for this is given. Surprisingly, atomic force microscopy images, taken at room temperature, do not show any helical structures in *Phanaeus kirbyi*.

Keywords: *Scarabaeidae*, *Notiophanaeus*, jewel beetles, chitin, epicuticle, Bouligand structure, Bragg´s law, cryogenics, cryobiology, RGB, AFM, SEM, metallic color, interference, iridescence, circular polarizer, birefringence, wetted surfaces.

Resumen

Analizamos el cambio de color del exoesqueleto del escarabajo Phanaeus kirbyi cuando se enfría de temperatura ambiente a 5K. Se esperaba un corrimiento hacia el azul de unos 10nm, debido a la contracción de las estructuras helicoidales que serían las responsables de la reflexión circular de Bragg y la consecuente luz polarizada circularmente observada en el insecto. El cambio esperado a azul no se puede confirmar, ya que el análisis RGB mostró colores no espectrales. Sin embargo, se puede observar un oscurecimiento del exoesqueleto con el enfriamiento y se da una explicación didáctica de varias posibles causas físicas para esto. Sorprendentemente, las imágenes de microscopía de fuerza atómica, tomadas a temperatura ambiente, no muestran estructuras helicoidales en Phanaeus kirbyi.

Palabras clave: Scarabaeidae, Notiophanaeus, escarabajos joya, quitina, epicutícula, estructura de Bouligand, ley de Bragg, criogenia, criobiología, RGB, AFM, SEM, color metálico, interferencia, iridiscencia, polarizador circular, birrefringencia, superficies mojadas.

I. INTRODUCTION

The phenomenon of structural color is ubiquitous among plants and animals in Nature [1]. For example, for many beetles their iridescent color does not originate from dyes, but is caused by light interference that allows only one wavelength to be reflected in a given direction, from their exoskeletons [2].

Due to its origin, structural colors usually show polarized light. The study of the polarization of light is one of the foundations of Physical Optics. Also various aspects of Quantum Mechanics can be experimentally analyzed and didactically studied using polarized light [3]. Unpolarized light reflected by a transparent dielectric becomes fully polarized when the reflected beam is perpendicular to the refracted one, at the so-called Brewster angle. Another way of transforming unpolarized light into fully polarized light by reflection occurs in cholesteric liquid crystals [4]. Michelson, in 1911, also observed that certain beetles, when illuminated by unpolarized white light, reflected circularly polarized monochromatic light [5]. It was later discovered [2] that the process how this occurs is similar to that of cholesteric liquid crystals: circular Bragg reflection [4]. So if we incident polychromatic light normally to a material, arranged in layers, where each layer is optically anisotropic and whose optical axis forms a helicoid with pitch p, the reflected light will have the following properties [4,6]. The reflected light will be quasi-monochromatic (if the anisotropy is small, $\Delta n/n << 1$) with wavelength $\lambda = n.p$ (for normal incidence), where n is the average refractive index of the anisotropic material and will be circularly polarized with direction equal to that of the helicoid.

Here we study the beetle *Phanaeus kirbyi* which reflects blue circularly polarized light when natural, i.e. unpolarized white, light falls on it. Due to the possible helicoidal structures that generate such kind of reflection, a change in the color of the beetle is expected when it is cooled from room temperature to 5K, as a function of the contraction of these helicoids.

II. MATERIALS AND METHODS

A male specimen of *Phanaeus kirbyi*, shown in figure 1, was cleaned with 70% etanol and fixed with Kapton tape to the sample holder of a Sumitomo RDK-408D2 dry cryostat, which has a fused silica optical window. With an achromatic quarter-wave plate and a tandem polarizer [6] we check that this beetle reflects (left) circular polarized light. The temperature was lowered from 300K to 5K in about 1 hour, under primary vacuum Throughout the experiment, no condensation on either the optical window or the sample was observed.

FIGURE 1. The beetle *Phanaeus kirbyi* analyzed is this study.

We photographed 3 distinct regions of the exoskeleton (3 times each), with a Nikon P510 camera, iluminated at ~60°, with a 250W Philips 13352 E/44 (IV) incandescent lamp at ~1m distance. Although this lamp is intended to heat large surfaces, we have to remember that heat is not a property of light, but is associated with material bodies. Therefore, although the lamp generates infrared radiation (which many people incorrectly associate with heat and transmit this false information to their students) it emits strong visible radiation with a continuous spectrum. It is this visible radiation that is the main cause, in this type of lamp, of the heating of bodies. As the cameras have a filter that blocks the near infrared before its sensor, the colors seen by the sensor mimic those actually seen by the human eye and the eventual infrared radiation present in the incandescent lamp is irrelevant in the analysis of the data. With Adobe Photoshop software the color was quantified by the RED- GREEN-BLUE (RGB) primaries, calculating the mean and standard deviation of 3x3=9 samples, for each temperature. The exoskeleton was analyzed at room temperature by atomic force microscopy (AFM) with a Shimadzu SPM 9.600 model, contact mode and also by scanning electron microscopy (SEM) with a Tescan Mira 3 model.

III. RESULTS AND DISCUSSION

A. General change in superficial color as the temperature decreases

The exoskeleton of the *Phanaeus kirbyi* beetle has what is called a structural color [1], if we assume it has a helicoidal structure analogous to *Chrysina gloriosa* [2]. In this case, it should have the pitch of this helicoid decreased due to the contraction that occurs with decreasing temperature. This is because the pitch is the empty space between two consecutive turns of the helicoid and we know [7] that empty spaces or "holes" in the bodies behave, with respect to thermal expansion and contraction, as if they were the bodies themselves. Due to the phenomenon known as circular Bragg reflection [2, 4, 6], these structures reflect light, under normal incidence, that has wavelength λ equal to

$$\lambda = np, \qquad (1)$$

where p is the pitch of the helicoid and n is the average refractive index of the material. The reflected light will be circularly polarized, with the same direction as the helicoid. Therefore, as it is cooled and the pitch of the helicoid decreases, the light reflected by the helicoid should have a blue shift, decreasing its wavelength. To evaluate this deviation, suppose that the blue color of the beetle at room temperature has a wavelength of approximately λ = 460nm. The coefficient of expansion of chitin is worth $\alpha = 7.3 \times 10^{-5}$ °C^{-1} [8, 9]. Assuming that an eventual thermal variation of the refractive index of chitin is much smaller than that due to the contraction of the helicoid pitch, we can consider its refractive index constant (n = 1.65 in blue [10]). In this case, with the decrease of about 300K in temperature, the color reflected by the beetle exoskeleton should shift to blue about *10nm*, approaching λ = 450nm.

Given the wavelenghth of a monochromatic spectral colors it is possible to establish a correlation, at least approximate, with the additive color model called RGB (from "red", "green" and "blue") [11, 12, 13]. Note that the opposite is not true, because not all RGB combinations have a spectral match. For instance, those where the percentage of red, green and blue are equal range from "white" (100% for the three colors), among various “grays”, to "black" (zero per cent for the three colors). These are not spectral colors although they belong to RGB system.

A spectral color shift as we consider, from $\lambda=460nm$ to $\lambda=450nm$, would be clearly discernible in the RGB pattern. However, the experimental RGB values from *Phanaeus Kirbyi* resulted in non-spectral colors. The problem may be caused by the lighting. Perhaps the incandescent bulb is not hot enough and thus the spectral distribution of its light has too little blue radiance. Therefore it was not possible, even approximately, to ascertain whether there was the expected blue-shift in the color of the beetle's exoskeleton as its temperature is lowered.

Scanning electron microscopy (SEM) analysis shows that *Phanaeus Kirbyi* has small polygonal protuberances on its exoskeleton, on the order of 10μm (see fig.4). This is similar to those found in *Chrysina gloriosa* [2]. However, unlike the latter, the atomic force microscopy (AFM) images (see fig. 3) do not show the heliciodal structures that one would expect from a beetle reflecting circularly polarized light.

B. Darkening of superficial color as the temperature decreases

It can be seen from figure 2 that there is a decrease in the percentages of all colors (red, green and blue) as the temperature decreases. This corresponds to an overall darkening of the exoskeleton, as zero percent of the three colors correspond to the "black" of the given RGB system and 100% of the three colors correspond to the "white". We discuss, in a pedagogical way, some physical phenomena below and use this attempt to explain such exoskeleton darkening.

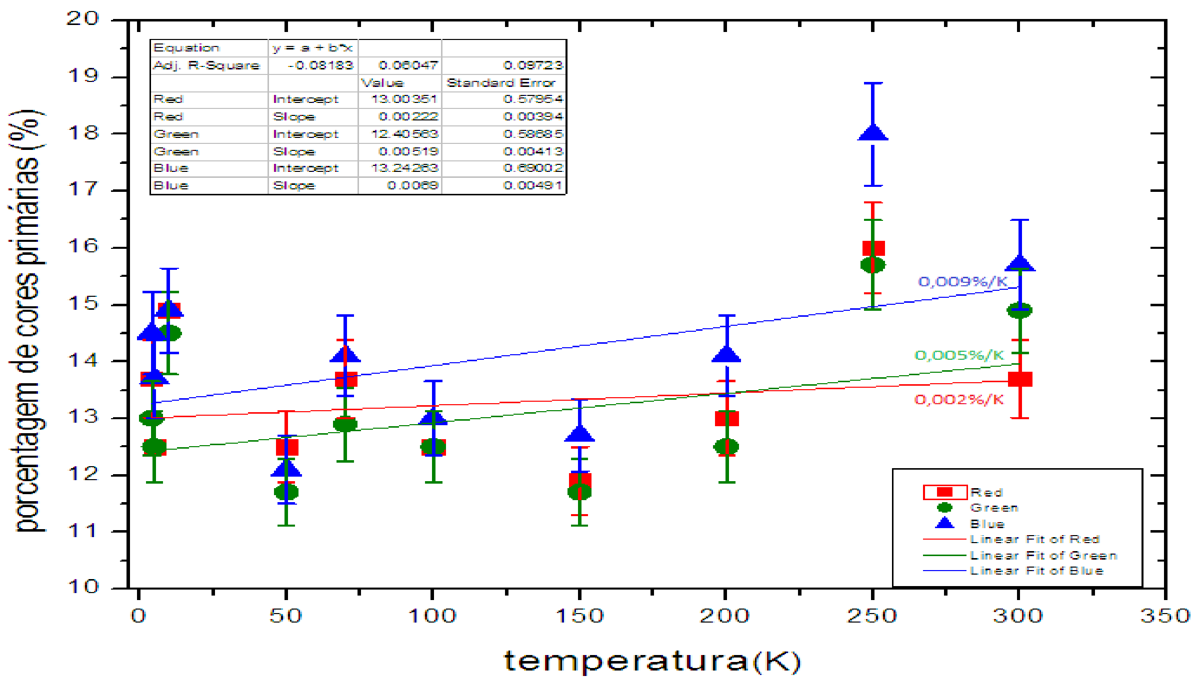

FIGURE 2. Variation, with temperature, of the color of the exoskeleton of *Phanaeus kirbyi*. RGB color system, with the linear adjustment of the percentages of the primary colors red, green and blue.

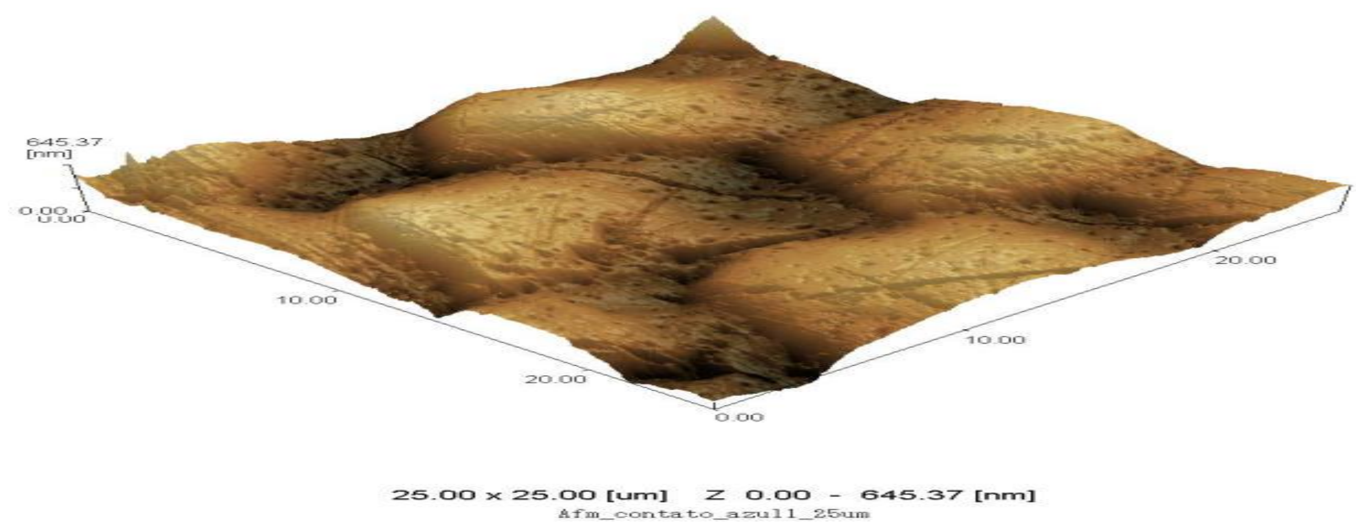

FIGURE 3. Image of the room temperature exoskeleton of *Phanaeus kirbyi* from contact mode of atomic force microscopy (AFM). Helical structures are not evident, at the surface.

Ideal black bodies get darker when they cool down, since the intensity of radiation at all frequencies varies with the fourth power of the absolute temperature of the body, according to Stephan's law [14]. However, even if we can approximate the beetle exoskeleton to a black or gray body at ambient temperatures or below, the emitted radiation is overall at frequencies far below visible light (Wien's law) and does not contribute to the darkening observed. Therefore, this darkening must be related to what we call albedo. This quantity is usually defined for unpolished bodies, where diffuse reflection prevails over specular reflection. In this case, the ratio between the intensity of diffusely reflected light and the incident one is what may be called albedo [15].

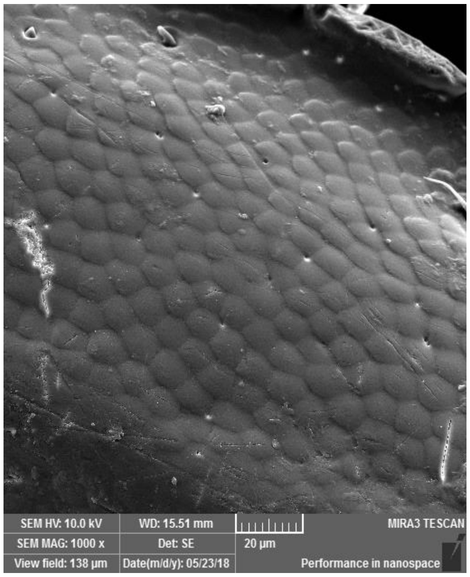

FIGURE 4. Microphotograph of the room temperature exoskeleton of *Phanaeus kirbyi* using scanning electron microscopy (SEM). Typical length of each polygon is about 10μm.

One possible explanation would be an interchange between diffuse and specular reflection of the beetle exoskeleton. As the temperature decreases there would be an increasing departure from an ideal diffuse reflection, i.e. Lambertian reflection [16]. The exoskeleton, like many unpolished materials (with respect to visible wavelengths), exhibits a mixture of specular reflection and diffuse reflection, with a strong prevalence of the latter. However, the irregularities of the surface, both vertically and horizontally, tend to decrease with temperature. This is due to the contraction of the materials as they cool. As the material itself, the "holes" in it also contract [7]. Similarly, the vertical variations between "valleys and mountains" will decrease with the thermal contraction of the material. Therefore, with the reduction of its temperature, an effect similar to that due to a polishing of the surface occurs. This will cause the mixture of specular and diffuse reflection to tend more and more toward the former as cooling occurs. Since the camera that photographed the exoskeleton was not at the particular angle of specular reflection, what was observed was only a decrease in diffuse reflection relative to those at the higher temperatures. During the experiment we did not pay attention to this possibility.

Therefore, it was not tested whether, in fact, at the particular angle of specular reflection there was a concomitant increase of light energy with the lowering of the temperature of the beetle. At any rate, although this reasoning is qualitatively plausible, it has its support compromised in a quantitative evaluation, as we will see below. Chitin, the main constituent of the exoskeleton, is an anisotropic material, with different thermal expansion coefficients for each direction. Moreover, these coefficients are not constant, but tend to increase in value with increasing temperature [8]. But let us take an upper limit, that is, let us take the highest value of the various anisotropic coefficients and at room temperature [8, 9]:

$$\alpha = 7.3 \times 10^{-5}\ °C^{-1}. \qquad (2)$$

This is a large coefficient compared to usual materials, such as most metals [17]. Despite this, the linear shrinkage suffered with the 300K-reduction in temperature in our experiment would be only 2%. This is not sufficient for a significant increase in the polishing of the surface and its concomitant specular reflection. For example, the flatness, one of the most important technical parameters that demonstrate the polish quality of a surface, would improve very little: the average distance between "valleys" and "mountains" would decrease by only 2%. As a comparison, observe that commercial mirrors, for common use, have flatness of 4 to 6 λ (where λ is the wavelength of light in the visible region; λ = 632.8nm is of common use) while a technical mirror needs to have at least a flatness of $\lambda/2$ or $\lambda/4$ [18]. In summary, the effect of darkening, with lowering temperature, by reduction of diffuse reflection at the expense of increased specular reflection, if any, would not be significant.

Another explanation for the decrease in the albedo of the beetle's exoskeleton with decreasing temperature would be changes in the refractive index of its components. One can establish an approximate relationship between the refractive index n and the polarizability α of a dielectric through the Lorentz-Lorenz equation [19]:

$$\frac{n^2-1}{n^2+2} = \frac{4\pi}{3} N\alpha, \qquad (3)$$

where N represents the average number of scattering molecules per unit volume. In the case of apolar molecules, polarizability α is not directly temperature dependent [20]. However, N and α depend on the density of the the material. Therefore, due to the contraction of the dielectric upon cooling, there is an indirect variation of the refractive index n with temperature. Since N increases but α in many cases decreases with density, the refractive index n has a behavior that is not easily predictable with temperature. For example, of the five glasses analyzed by Waxler [21], four decrease their refractive index with decreasing temperature and one increases. Hence, one cannot easily predict how the refractive index of chitin will change with decreasing temperature.

Insects, in general, have a thin coating of wax over their exoskeletons, called an epicuticle [2, 22]. The main components of waxes are alkanes, which belong to the class of hydrocarbons [23]. As seen above, the behavior of the refractive index of dielectrics with temperature is varied. However, experimentally, it is observed that there is an increase in the refractive index as the density of the alkanes increases [24]. Therefore, the wax surrounding the beetle would have an increase in its refractive index as the temperature is lowered.

On the other hand, there is an explanation as to why certain rough objects become darker when wet [25]. The light coming from diffuse reflection on the rough surface suffers a total internal reflection at the air-water interface from the aqueous film surrounding it. This ultimately decreases the light returning to the environment, making the wet object darker. Similarly, increasing the refractive index of the wax film surrounding the rough chitin that forms the beetle's exoskeleton would increase the total internal reflection with respect to that which already occurs at room temperature.

This would decrease the overall return of light to the environment, darkening the insect as we lower its temperature.

Of course chitin itself, undergoing contraction, has its refractive index changed with temperature. However, although we do not know this particular behavior of chitin, we will assume in this reasoning that it would be of second order.

In summary, we believe that the most likely explanation for the observed darkening of the exoskeleton of *Phanaeus kirbyi* with the lowering of its temperature is due to the increase in the refractive index of the wax-epicuticle that covers it.

IV. CONCLUSIONS

We believe this is the first time that a beetle has been analyzed at temperatures as low as 5K, although it is known that certain species can withstand cold of around 173K [26]. Since our beetle reflects circularly polarized light, it should have helicoidal structures in its exoskeleton, which would contract as the temperature drops. This would result in a blue-shift of about 10nm in its color. We could not confirm this expected deviation, apparently due to the limitations of the RGB system or the problem may stem from the type of lighting used. Also, the helicoidal structures of *Phanaeus kirbyi*, if they exist, were not visible in our atomic force microscopy analysis. The absence of the these traits was surprising, since this is the only known process for generating circularly polarized light in beetles [2]. Thus, it seems that there is a second, yet to be clarified, process for circularly polarized reflection in these animals.

Although a spectral change in the color of the exoskeleton cannot be confirmed, the experiment shows that there is a darkening of the exoskeleton as the temperature decreases. The cause of this seemed to be due to an increase in the refractive index of the wax that forms the beetle's epicuticle as the temperature drops. This would increase the total internal reflection of light diffused by the exoskeleton itself, darkening it in a similar way to a rough object that has been wet. In order to be didactic, we discuss various optical phenomena involved.

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