Switchable reflector in the Panamanian Tortoise Beetle *Charidotella egregia* (Chrysomelidae: Cassidinae).

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(Dated: July 19, 2007)

The Tortoise beetle *Charidotella egregia* is able to modify the structural colour of its cuticle reversibly, when disturbed by stressful external events. After field observations, measurements of the optical properties in the two main stable colour states and SEM and TEM investigations, a physical mechanism is proposed to explain the colour switching on this insect. It is shown that the gold colouration (rest state) arises from a chirped multilayer reflector maintained in a perfect coherent state by the presence of humidity in the porous patches within each layer, while the red colour (disturbed state) results from the destruction of this reflector by the expulsion of the liquid from the porous patches, turning the multilayer into a translucent slab that leaves a view on a deeper-lying pigmental red substrate. This mechanism not only explains the change of hue but also the change of scattering mode from specular to diffuse. Quantitative modelling is developed in support of this analysis.

PACS numbers: 42.66.-p, 42.70.Qs, 42.81.-i, 42.81.Qb

I. INTRODUCTION

It has been observed that some living organisms rapidly adapt their appearance in response to changes in the environment. The immediate threat of, for instance, a predator attack can trigger reactions, like suddenly showing unexpected coloured body parts. This is very common among flying insects, which can hide and suddenly show brightly coloured or conspicuously patterned hindwings, a strategy to confuse their predators and delay adverse actions [1].

More complex transformations are sometimes observed. Among terrestrial animals, one of the best known examples is probably the chameleon, some species of which (for instance *Chamaeleo chamaeleon*) are able to modify the colouration of their skin [2, 3], partly according to their mood, partly as a cryptic adaptation to their surroundings. In that case, the change of colour is explained by the migration and volume change of special cells (chromatophores) dispersed in the skin below the transparent outer layers. With reddish "erythrophores", yellow "xanthophores", and blue "iridophores", the chameleon's skin can, by adjusting the absorption efficiency of each type of chromatophore, displays a large range of hues and colour patterns. The presence of melanin in deeper layers also allows dark or light colours to be produced, according to the diffusive power given to the chromatophores. Another recently discovered reptile, the Kapuas mud snake (*Enhydris gyii*) from the Kalimantan area of the Borneo rainforest[4], switches from red-brown to white, but the underlying mechanism has not yet, to our knowledge, been fully elu-

FIG. 1: (Colour online) Adult *Charidotella egregia*. This leaf beetle, (8 mm in size) is a representative of the large family Cassidinae. The insects in the family Cassidinae are often called "Tortoise beetles," because they have a wide, hard and strong armour, which includes the wing cases, the pronotum and the head cuticula. It extends widely, beyond the insect's periphery, providing a very efficient shield against attempts to grip the insect when it is lying flat on a supporting leaf (photo Marcos A. Guerra, with permission).

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FIG. 2: (Colour online) Adult *Charidotella egregia* switching from gold (above) to red (below). The timing of the whole process is variable, but a typical minimal conversion time is 1 min 30 sec. This transformation was triggered by touching the wing cases with a hard rod, plausibly simulating a missed attempt at predation.

cidated. Numerous octopuses, squids and cuttlefishes are able to change their skin colour – most famously, species of the blue-ringed octopus, *Hapalochlaena spp.* The squid *Loligo pealei* is, likewise, able to modify its skin hue for camouflage and intraspecific communication [5]. It has been recently discovered that a species of octopus from Indonesian waters is not only capable of changing its skin colour, but also seems to have evolved a very high degree of variability, allowing it to mimic the appearance of poisonous creatures, including their colouration patterns and body shapes [6].

Among insects, reversible changes of colouration have been described in several species, such as the phasmid *Carausius morosus* [7–9], the larvae of the dipteran *Corethra* [9–12], the grasshopper *Kosciuscola tristis* [13, 14], a number of damsel flies [15–22], and some Coleoptera [23, 24]. Many of these examples show passive colouration changes due to diurnal changes of the atmospheric hygrometry, but the Cassidines considered here are special because they undergo a more active change of colour, controlled by the insect itself, as a reaction to events occurring in the environment. A review considering many cases of Tortoise beetles (Chrysomelidae: Cassidinae) can be found in a paper by Pierre Jolivet [25].

The early works on Cassidines led to the realization that the colouration change was structural, involving the selective reflection of light by a thick multilayer, in the outer layers of the transparent armour. Quantitative physics, however, has not yet contributed its share to the precise reverse engineering of this structure and the mechanism of colouration changes. The widespread assumption – the "hydraulic theory" [23, 25, 26] – is that the physical characteristics of the multilayer (refractive index and/or thickness of the layers) are modified under the pressure of a fluid, to produce the colour changes. However, we will see that the variable beetle under study here does not use this widely assumed mechanism.

The insect that will be the subject of the present work (see fig. 1) is *Charidotella egregia* (Coleoptera: Chrysomelidae: Cassidinae). Our choice of this insect was influenced by the very strong and rather rapid change of appearance observed with this species and by intriguing questions raised by the observation of the phenomenon. One of these is that the modification not only affects the hue, but also the type of surface scattering : the appearance changes from a "metallic" sheen into a "matt", or "diffusive", aspect. This change, from specular reflection to wide-angle diffusion, calls for an improved analysis, which led us to need new, accurate optical measurements, scanning electron microscopy investigations, detailed modelling of the structure and the confirmation of the interpretation by numerical modelling as well. Since the effects to be observed only occur with living animals and since these animals can only feed on a specific plant, it was mandatory to make at least some of the observations and measurements in or near the insect's habitat. The collection of specimens and many of the optical measurements were developed at the Smithsonian Tropical Research Institute in Panama.

II. OBTAINING SAMPLES

The Golden Tortoise beetle *Charidotella egregia* (Boheman 1855) (Chrysomelidae: Cassidinae) is reported to feed specifically on the vine *Ipomoea lindenii*, and its presence can often be detected by observing recent feeding damage on the leaves of this plant. For our investi-

gations, we found the host-plant in a place named Cerro Galera[27] near Panama City and, in early September, we were able to find 12 adult specimens. We noted a significant number of larvae and pupae at the site in that period, which was encouraging with regard of the condition of the population and reassured us that capturing a few individuals for our investigations was innocuous.

These specimens were kept alive, maintained in individual transparent boxes to ease the preparation for optical measurements. They were provided with an appropriate leaf from their host plant. The moisture in each box was maintained at a fairly high level, in order to prevent the leaves (which were renewed nearly every day) desiccating rapidly and attempting to reproduce the hygrometry of their habitat. The insects were able to live for several weeks under these conditions.

III. APPEARANCE TRANSFORMATIONS

In the case of the Tortoise beetle *Charidotella egregia*, the change of colouration is observed on the central part of its dorsal armour, excluding the flat, transparent, peripheral extension that has evolved to match the leaf surface closely. The transformation from gold to red can be initiated by touching the insect with some hard stylus, strongly enough to displace the insect sitting on its leaf by a few millimeters. This excitation can be compared with a blow from a bird in a missed predation act. After less than two minutes, the change in appearance, from gold to red is completely achieved. At the same time as the hue is changing, the metallic aspect disappears and the surface under the transparent armour becomes more diffusive. Fig. 2 shows the details of the appearance of the insect during this transformation. As can be seen, the colour does not change uniformly. The change from gold to red first occurs at the periphery of the wing cases and on the external and central parts of the prothorax, leaving zones of high reflectance. The low-saturated red state is the "disturbed" state, reached when the insect has been disturbed, while the gold state is the insect "rest" state, which is recovered after a long quiet stay on, or near, the food-plant. For *Charidotella egregia*, the red state has also been observed in its natural habitat, during a heavy tropical rainstorm (at that moment, all adults encountered had turned red). The gold state has repeatedly been observed in adults sitting on the top side of a leaf, in a bright sunshine. It appears that "newborn" adults emerge from the pupa in the red state and that, at maturity, both the male and the female are in the gold state when mating.

Another important observation, for the argument developed in the present paper is that the gold appearance is lost when the insect is dead and dry. The elimination of moisture from the cuticle leads to a reddish appearance close to (but not exactly the same as) the "red" colour seen in the disturbed state of the Cassidine.

IV. OPTICAL PROPERTIES

Measuring the optical properties of a susceptible living animal is somewhat tricky. In the gold state, for instance, one could not manipulate it (for instance to hold it still under the probe of the spectrometer), as it would easily change state before, or while the measurement was being carried out. Thus, in order to measure the reflectance spectrum of the gold state of *Charidotella egregia*, eight adults were installed in individual transparent boxes, with a fresh leaf on the floor. In the morning, all individuals were usually found resting in the gold state, some of them sitting still on the upper side of the leaf. These were subjected to a measurement directly inside their box, avoiding any disturbance. An Avaspec 20048/2 fibre-optic spectrophotometer was used, with a bifurcated probe designed to collect the emergent light on the same path as the incident illumination, in an exact backscattering geometry. The probe containing the terminal parts of the optic fibres (a steel tube terminated by a lens) was passed through a fine textile net, shaped as a cone, attached to the periphery of the box top cover, so that it could be moved freely inside the box, without allowing the flying samples to escape. This simple technique enabled us to take many spectra of the living insect in both states of colouration (gold and red) and to show intermediate steps in the colour conversion.

Fig. 3 shows typical results. The solid curves (labelled "Gold" and "Red") correspond to well-defined gold and red stable states. "Gold" describes a state where the cuticle effectively contains a dielectric reflector[28], which gives the surface a metallic gold aspect. "Red" denotes

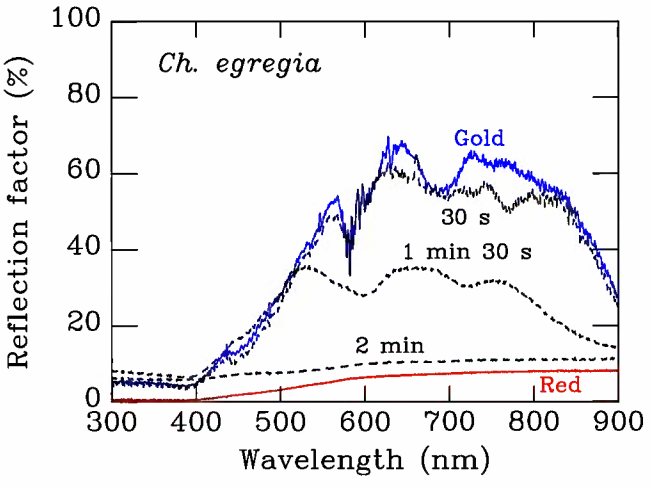

FIG. 3: (Colour online) Reflection factor from the back of *Charidotella egregia*. The solid lines labelled "Gold" and "Red" are, respectively, the reflectance from the gold and red states. The dashed lines in between are intermediate reflectances, measured 30 seconds, one and a half minutes and two minutes after applying a stressful disturbance. The measurement is performed in a backscattering geometry, in the direction of the normal to the cuticle surface.

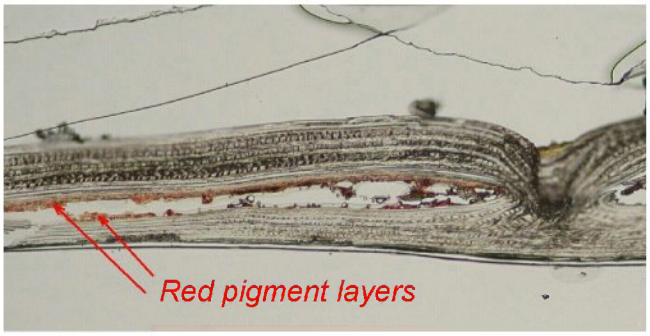

FIG. 4: (Colour online) Cross-section of the elytron of *Charidotella egregia*, indicating the location of the red pigment. The outer surface of the elytron is at the top of the section. The red contribution comes from a deep layer, well below the gold Bragg mirror (not perceptible at this scale).

a state where the surface has a diffusive red aspect that we consider to be the "disturbed" state, because its appearance can be triggered by some external disturbance. The gold colouration is metallic, which means that the reflectance is high and that the scattering is predominantly specular. This suggests the presence of a multilayer, but in this case, the lack of colour change with the incidence angle and the colour desaturation more precisely indicates a broadband chirped dielectric mirror. The reflection factor remains high between 500 nm and 900 nm, which means that it covers the visible, with some near-infrared, but lacks a blue component : hence the yellow hue of the "gold" colour, which does not change with viewing angle (thus using a chirped geometry reminiscent of that occurring in *Chrysina resplendens* [29], however with an isotropic material for each layer, so that the circularly polarizing properties of the latter does not occur). The spectral range is achieved by several reflection bands, which can be roughly located at the wavelengths 560 nm, 640 nm and 810 nm. This, again, suggests that the gold dielectric mirror is chirped (with layer thicknesses varying with their depth in the cuticle) and that it can be seen as a stack of more elementary dielectric mirrors, each optimized for a specific wavelength. This will be directly confirmed by nano-morphology studies, which will be the subject of the next section. The red colouration appears to be spectrally unstructured and can be described as a vermillion hue, showing a uniform mixture of red, orange and a weaker addition of yellow. This colouration results from incoherent scattering of non-absorbed light: a process normally associated with the presence of pigments.

When the change from gold to red is activated by stressing the insect, the reflectance spectrum is progressively modified. The amount of the insect's surface covered by the gold reflector diminishes, in favour of the red colouration. The curves shown in Fig. 3, labelled "30 s", "1 min 30 s" and "2 min" correspond to reflection factors, measured at the centre of the remaining gold area, at the indicated time after the stress application. After 30 s, the reflectance is not significantly changed, but after 1 min and 30 s, we note both a significant reduction in the reflection intensity and a blue shift of the different components of the broadband spectrum (now perceived, respectively, near 520 nm, 650 nm, and 750 nm). We note that the blue intensity near 450 nm is not weakened, but the other components of the spectrum (greenish yellow, orange and red) are reduced by nearly 50%. The reflection spectrum decreases significantly in the near infrared, but still extends over a large part of the visible range. The relative weight of the blue component increases because of the decrease of the yellow-red part, with the consequence that the colour of the reflector is noticeably desaturated. Indeed, even with the naked eye, the colouration of the dielectric mirror is observed to turn from its initial gold colour to a white silver hue, before disappearing. After about two minutes, the gold reflector has disappeared and the entire surface reaches a diffusive red colour (a specular reflection generates highlights, i.e. sharp images of light sources, as on metallic surfaces, while a diffusive colour does not).

Contrary to what could be expected from the "hydraulic" mechanism, the spectrum does not *shift* rigidly from yellow to red (something that would occur if, for some reason, the layers were only to increase their thickness or increase their average refractive index, as the result of a fluid injection). Rather, as the transformation proceeds, the high gold reflectance *disappears* and what remains is a broad, unstructured, red diffusion. The question is less about how the dielectric mirror is modified to shift the selection from a yellow to a red hue, than about how this mirror destroys itself, to leave a view of the red pigmentary material which is found beneath this multilayer. The location of this red layer is shown in Fig. 4, on a thin slice of an elytron cut along a cross-section, and viewed with a transmission optical microscope. The elytron is hollow, and the red pigment lies at the edges of

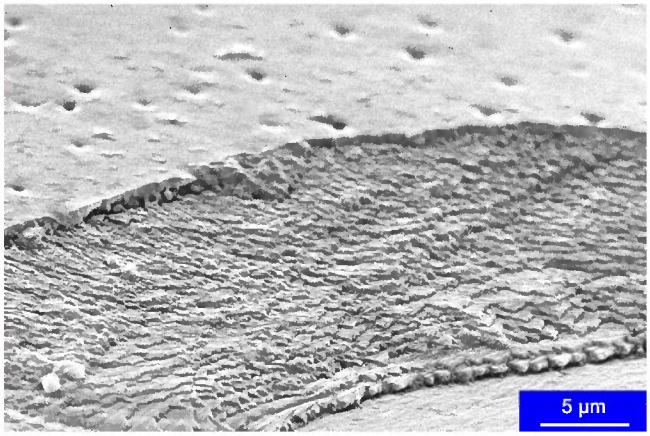

FIG. 5: (Colour online) Scanning electron microscope view of the dry reflector, which produces the gold colouration. The obliquely broken exocuticle reveals the layered structure clearly, with layer thicknesses that increase proximally (i.e. towards the inside of the insect).

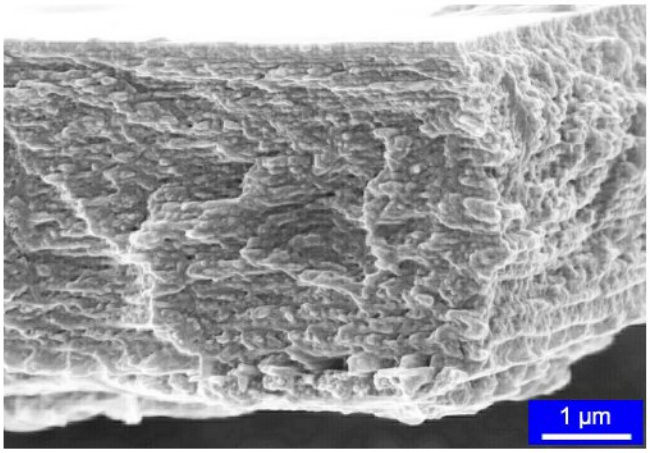

FIG. 6: (Colour online) In this sample for SEM, the fracture occurred normally, so that the thickness of the layers forming the gold reflector can be easily measured, in spite of the irregularity of the section surface.

the cavity, well below the dielectric mirror that produces the gold reflectance.

In the following section, we try answering this question by considering the morphology of the cuticle to a resolution of a few nanometers.

V. NANO-MORPHOLOGY

Physical colouration is basically due to the multiple scattering of light by an inhomogeneous medium. For red-orange visible light, for instance ($\lambda = 600$ nm), in a medium with an average refractive index $\bar{n} = 1.5$, a typical distance between scatterers in the structure should be [30]

$$d \approx \frac{\lambda}{2\bar{n}} = \frac{600 \text{ nm}}{2 \times 1.5} = 200 \text{ nm} \qquad (1)$$

This distance decreases for shorter wavelengths or higher average refractive indexes. A resolution better than 10 nm is then required to study such structures and, consequently, we submitted transverse sections to a detailed examination with the scanning electron microscope (SEM) and the transmission electron microscope (TEM). The samples were prepared by cutting a piece of desiccated elytron in liquid nitrogen and covering it with a 15 nm thick gold layer. To begin the investigations, we searched for a spot showing a very oblique fracture. The idea of searching for the golden reflector with the SEM is not completely straightforward because, once desiccated for observation, the metallic gold appearance of the sample has effectively disappeared, which means that, at best, we can only expect to observe a "damaged" Bragg mirror. However, surprisingly, one finds this reflector apparently intact, in the form shown in Fig. 5. The topography of the terraces indicates that the exocuticle is a stack of hard layers with mechanically weaker junctions. The geometry of the structure provides a "chirped" multilayer stack, with layer thicknesses that decrease in the distal (outward) direction. Strangely enough, the number of periods is not always the same for samples taken from different locations of the insect's armour : the number of periods actually varies from about 20 to 40, without affecting the resulting visual appearance too much. In Fig. 6, the same type of multilayer is seen with the fracture normal to the cuticle surface. The chirped structure is confirmed, with a thickness variation such that the thicker layers have more than twice the thickness of the thinner layers. The fracture is highly irregular, which indicates that the material which forms the layers is, also, likely to be structured in the directions parallel to the multilayer's interfaces. Such lateral structuration is often encountered in the cuticles of Coleoptera [31] : for instance, the presence of parallel bars of chitin wrapped in proteins can explain the anisotropy of the layers, as needed to produce circularly polarized light from the reflection of unpolarized illumination, as observed in many

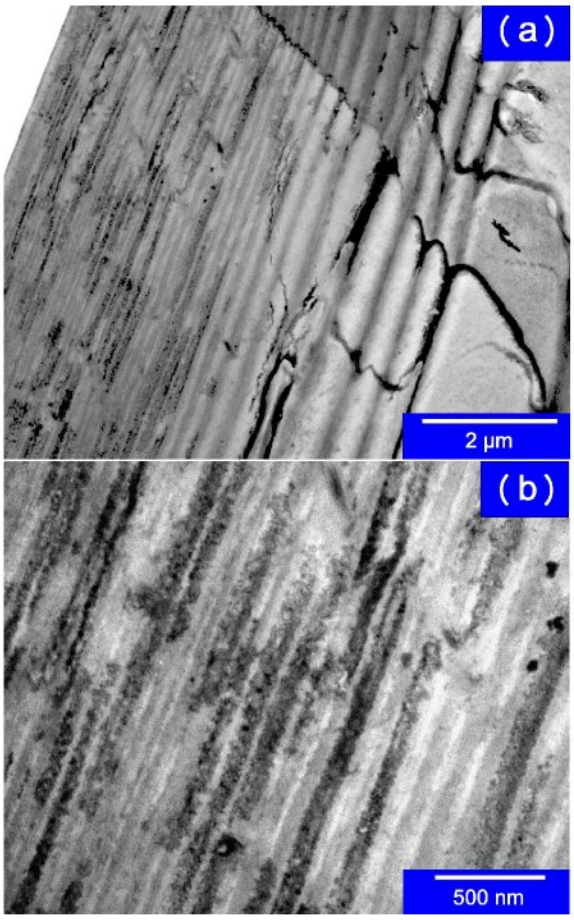

FIG. 7: (Colour online) Transmission electron microscope view of a thin slice of the cuticle of *Charidotella egregia*, showing irregularities in the material density, parallel to the layers. This finite-size flat zones oriented parallel to the surface can be described as porous patches distributed irregularly in each of the layers of the dielectric mirror.

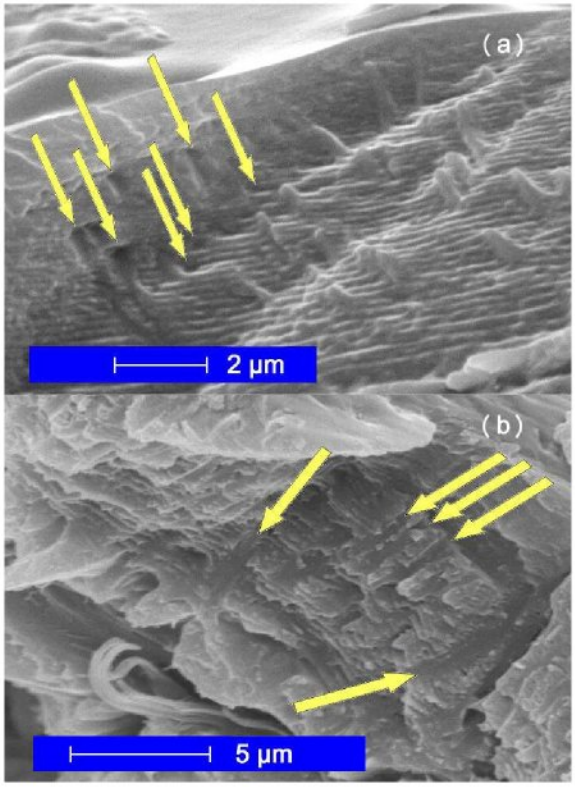

FIG. 8: (Colour online) Scanning electron microscope view of a severely damaged part of the cuticle of *Charidotella egregia*, showing channels running through the optical exocuticle. It seems likely that these channels bring (or remove) fluid into (or from) the patches in the colouring multilayered exocuticle.

Cetonniidae (see, for instance, Berthier [26] and references therein and [32]). However, in the actual state observed, nothing indicates that any polarization effects would be of importance in the case of *Charidotella egregia* and the structuration giving rise to the fracture roughness is not related to any obvious regular organization.

The multilayer reflector shown in Fig. 6 has a rather simple, chirped, structure, which can be understood as a stack of three Bragg mirrors, assembled on top of each other. The first one, at the cuticle surface, contains 12 layers, with an average thickness of 186 nm. Assuming a refractive index $\bar{n} = 1.5$ (slightly lower than that of chitin in order to account for some porosity), we can predict the spectral selectivity of this mirror : the dominant reflected wavelength is [30]

$$\lambda = 2\bar{n}d = 2 \times 1.5 \times 186 \text{ nm} = 558 \text{ nm}. \qquad (2)$$

This is a yellowish-green mirror that accounts for the peak (near 560 nm) at the short-wavelength edge of the "gold" reflection band shown in Fig. 3. The next set of layers also contains about 12 layers, providing a stack with an average period of 213 nm. As before,

$$\lambda = 2\bar{n}d = 2 \times 1.5 \times 220 \text{ nm} = 660 \text{ nm} \qquad (3)$$

and we are now in the red part of the visible spectrum. The second mirror is a rather thin (desaturated) red mirror, which can also explain the bump in the reflectance spectrum, near 640 nm in the reflection band in Fig. 3. Finally, the 8 next layers in the proximal (inward) part of the cuticle show an average layer thickness of 270 nm, which corresponds to a dominant reflected wavelength of

$$\lambda = 2\bar{n}d = 2 \times 1.5 \times 270 \text{ nm} = 810 \text{ nm}, \qquad (4)$$

at the edge of the infrared. This can be related to the broad component near 800 nm in the "gold" reflection band of Fig. 3. The simple analysis developed here shows that the cuticle multilayer seen at the surface of an elytron with electron microscopy has the dimensions required to explain the metallic reflection band observed in the "gold" state of the insect.

TEM reveals much more of the dielectric mirror layers. Ultra-thin sections of elytra, embedded in araldite, have then been cut in order to better observe the dry reflector cross-section. Fig. 7 shows the internal structure of the exocuticle at two different magnifications. At a magnification of about 10000× – Fig. 7(a) – the entire multilayer thickness is visible and each layer can easily be discriminated and characterized geometrically. At a larger magnification – Fig. 7(b) – the internal structure of the layers is revealed better. The density of the material in each of the layers can easily be perceived: the new fact is that each layer clearly shows inhomogeneity, i.e. variation of density in the directions parallel to the layers. This can be best described as "porosity patches" lying within the layers of the dielectric mirror. The presence of these patches can explain the reversible destruction of the dielectric mirror, if we assume that the porous regions can rapidly switch between "humid" and "dry" conditions. All periods of the Bragg mirror actually contains two layers : the first is thick and takes most of the period thickness and varies in density because it contains the porosity patches; the second is much thinner and appears to be

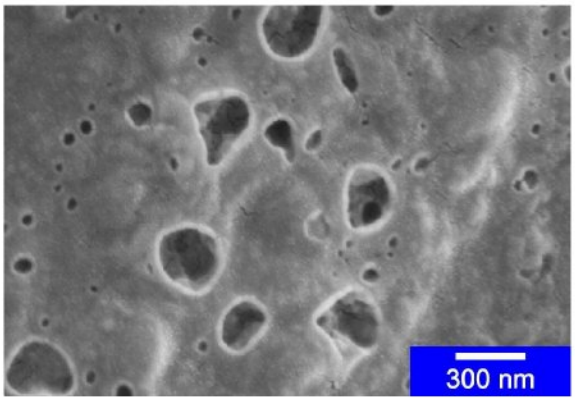

FIG. 9: (Colour online) Microwindows seen on the surface of unbroken elytra. These structures could accelerate exchanges of vapours between the inner volume of the optically active exocuticle and the outer atmosphere. However, no proof of a relation of these structure with the channels or the porosity patches could be brought by the present work.

made of an homogeneously light material (as can be seen clearly in the images in Fig. 7) and constitutes the junction between the "patchy" slabs. The porosity patches have the thickness of the thick layer component, occur at random lateral locations in this layer and assume a lateral size which varies between 300 nm and several μm.

The presence of porosity patches gives a clue for understanding the physical mechanism of the colour change of the insect. The two states of colour can be related to the states of hygrometry of the exocuticle. The porous patches will show a different refractive index when the pores are filled with liquid or empty. The fluid transport to the porous patches requires a network of channels in the material which constitutes the elytron, including the very fine optically active exocuticle. Fig. 8 confirms the presence of such a network. In Fig. 8(a), an oblique fracture of the exocuticle reveals these channels, indicated by arrows. Some of these channels manifest themselves as exiting cross-sections which appear at various places on the irregular surface, while others that have been sectioned parallel to their axis appear as longitudinal groves. The orientation of the channels is seen better in Fig. 8(b), where the optical exocuticle has been more severely damaged and several fracture orientations are present. It can be seen that some channels run horizontally (parallel to the layers of the Bragg mirror), and vertically (normal to the multilayer interfaces).

For completeness, one should also mention that SEM pictures of the unbroken surface of the insect cuticle show microscopic openings which, to our knowledge, are not seen in other families of insects (see Fig. 9). It is unclear whether these openings are actually connected to the network of channels or to the porosity patches, but we cannot rule out the possibility that these openings help physiological fluid exchanges between the exocuticle and the atmospheric gases. Pores are however frequent and always present in Coleoptera, where they indeed serve several purposes, including giving a way out to defensive compounds produced by specific dermal glands.

VI. COLOUR-SWITCHING MECHANISM

Developing a theory for this colour-switching effect is not completely straightforward, as this must account for a number of observations that are sometimes difficult to reconcile : (1) the insect can display two reversible states of colouration : a yellow (or more precisely "gold") state and a red state; (2) the gold state emphasizes selective specular reflection, while the red state is bright, but manifests itself as a wide-angle diffusion, lacking metallic aspect; (3) the change of colour occurs in patches which grow, following a well-defined sequence of areas on the dorsal cuticle; (4) the depth and the inside of the elytra is red; (5) the transformation from gold to red can be triggered by putting the insect under stress and takes about 1 min 30 s; (6) the transformation from red to gold can be as fast as gold to red, but the start of the transformation cannot be triggered and is unpredictable.

Furthermore, it is observed that (7) naturally dead insects are red-brown when dry (this is the case of most specimens in the museum collections); (8) if deep-frozen fast from the red state, the insect remains red when brought back (dead) to normal temperature; (9) if the insect is deep-frozen fast while in the gold state, it turns brown when frozen, but reverts to gold when brought back (dead) to normal temperature; (10) subsequently, dead deep-frozen insects, returned to gold, very slowly lose this metallic appearance and turn red as they become dry. For some time, they can still be forced back to gold in a very humid environment (for instance in contact with wet pads). These last observations are very important as they distinguish the interpretation presented in this work from that conveyed by the widely assumed "hydraulic theory".

A. Hydraulic theory

The change of colour from yellow (dominant wavelength 580 nm) to red (dominant wavelength 700 nm) could be understood as a change of the multilayer period thickness under the pressure of a fluid injected by the insect, as was proposed earlier for other Tortoise beetle species [23, 26]. The increase of the period a of a periodic multilayer with average refractive index $\bar{n}$ results in a proportional increase of the dominant reflected wavelength, according to the expression [30]

$$\lambda = 2\bar{n}a, \tag{5}$$

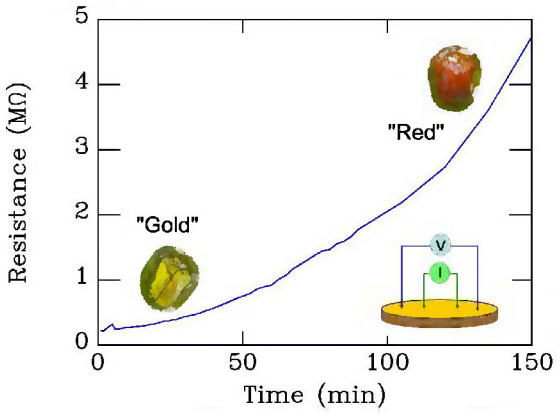

FIG. 10: (Colour online) Variation of electrical resistance of an elytron of *Charidotella egregia* between the "gold" (humid) state and the "red" state, measured with a Kelvin probe. The four-point, or Kelvin, probe method is a common way to measure the resistance of a flat conductor. Two of the probes are used to source current and the other two probes are used to measure voltage. Using four probes eliminates measurement perturbations due to the probe resistance and the contact resistance between the metal probe and the sample.

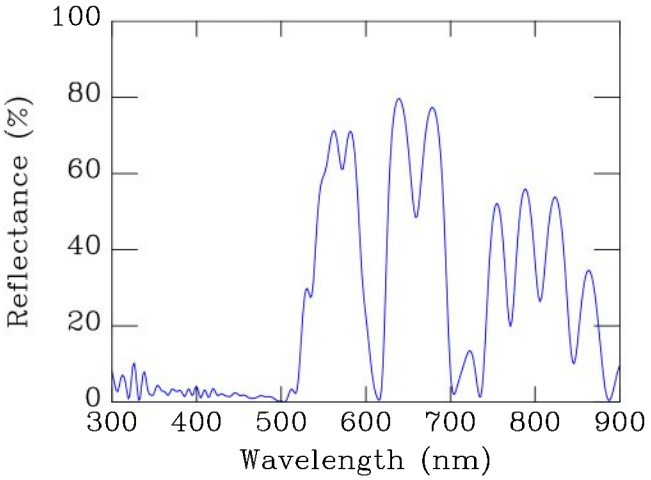

FIG. 11: (Colour online) Transfer-matrix reflectance calculation, which describes the spectral reflection from a chirped photonic-crystal film which stacks three Bragg mirrors, selecting yellow, red and infrared bands, as suggested by scanning electron microscopy data.

so, a 20% increase of the wavelength means a 20% increase of the layer thickness: a very large change requiring a remarkable elasticity of the layers. Furthermore, we note that, in this explanation, the humid state, with fluid injected in the structure, is the long-wavelength colouration, and this is the red state. The golden or yellow state is, by contrast, the dry state. This, however, contradicts many observations, including the fact that dry, dead insects turn red, and that red dry insects resulting from deep-freezing of an insect in the golden state can be reverted to the metallic gold appearance by exposure to humidity.

Another point against this widely believed interpretation is that it makes no distinction between specular reflection and diffusive scattering : the simple change of average refractive index and layer thickness does not change the translational symmetry of the Bragg reflector, meaning that we would expect a change from metallic gold to metallic red, instead of the diffuse red appearance which is actually observed.

B. Switchable mirror theory

In the present-work "switchable mirror" theory, the explanation is different : the dry specimen contains empty, porous patches, and this provides a set of scatterers that appear at random locations, with random lateral sizes, in the multilayer mirror structure. As the next section will show, this disordered scatterers distribution leads to a "white" diffusion, which means that the optically active multilayer is now a translucent layer, revealing a view of the red substrate. If a fluid is injected, the porous patches get filled and their refractive index becomes similar to that in the rest of the inner layer in all periods, and these inner layers become homogeneous and act as a flat layer with constant lateral refractive index. This rebuilds a near-to-perfect Bragg mirror, providing the gold colouration and, at the same time, the specularity which leads to a metallic aspect. In this theory, the "gold" state is the humid state, while the "red" state is the dry state. This, evidently, fits the observations better.

The red "pigment" in the layers under the Bragg mirror may not be static, but partially carried by the hemolymph which circulates in the bulk of the elytra. There are indications of such a possibility, in the fact that red-coloured layers have been observed in the depth of the cuticle in optical microscopy (see Fig. 4), and in relation with the observation that the red hue of a living disturbed insect is not exactly the same as the red-brown hue of a dead, dry insect. This origin of the red colouration is, however, still to be confirmed by biochemical investigations. We should emphasize that the red pigment is physically a blue-green absorber which subtracts only those wavelengths shorter than about 450 nm, and then should have no influence on the "humid" gold reflector engineering, even if some selective, short-wavelength absorption occurs in the optically active layers.

The difference between the earlier "hydraulic theory" and the present "switchable mirror theory" then is the contrasting interpretations of the "gold" and "red" states in terms of their liquid contents. The hypothesis that the gold (dominantly yellow) state is associated with the presence of moisture, while the diffuse red appearance is a "dry" state can be tested by a measure of the electric resistance of an elytron removed from a specimen in the gold state, as a function of time in a dry atmosphere, letting the elytron turn passively into the red state. In this experiment, the resistance was measured as a function of time, using Kelvin probe (four-probes) method, in order to avoid complications arising from non-ohmic contact resistances. The result is shown in Fig. 10. The elytron slowly changes colour, from gold to red as the bulk of the elytron, in contact with the colouring layers, loses its natural moisture, and this is accompanied by an *increase* in the elytron's electrical resistance. This experiment gives a qualitative additional support to the observation that the humid state corresponds to the metallic gold appearance, while the dry state gives the diffusive red aspect.

We should not, however, exaggerate the significance of this measurement, as the passive desiccation which takes place in this transformation, and the resistance recording, deal with the whole elytron, and not only the active optical layer. The time required to dry the whole elytron is much larger than the time required to make the gold reflector vanish, *in vivo*. This seems to indicate that an active mechanism must be present in the living insect to accelerate the desiccation of the optical layers without requiring the evacuation of the complete elytron structures. It was, unfortunately, not possible to conduct this experiment on a living specimen during a colour change, because the time required for a sample setup under the Kelvin probe exceeded by far the reaction time of the

insect.

C. Numerical simulations in support of the switchable mirror theory

The "gold" state, then, is the humid state, in which the porous patches are filled with a fluid, which makes the wide, partially porous layer in the Bragg mirror optically homogeneous. This turns the complex structure into a one-dimensional chirped photonic crystal which provides both specularity (hence the metallic aspect) and the appropriate spectral selection to explain the gold colour. Fig. 11 shows the result of a numerical simulation for a structure that matches the layer description in Eq. (2), (3) and (4). The calculated structure stacks three Bragg mirrors on top of each other. The "distal" mirror (at the multilayer surface) has 12 periods of 186 nm (25% of this thickness is a light junction material with refractive index 1.35 and 75% is a higher-index material with a refractive index 1.55); the intermediate mirror has 12 periods of 200 nm, with the same refractive index distribution as the distal mirror; the proximal mirror (inside) has 8 periods of 270 nm, again, with the same index distribution. A noticeable refinement in this calculation is the introduction of a pigmentary absorption for wavelengths shorter than 500 nm, in the form of an imaginary part in the dielectric constant ($\mathrm{Im}\,[\varepsilon] = 0.05$). The calculation uses a transfer-matrix approach, that solves Maxwell's equations with the full detail of vector multiple-scattering (for homogeneous isotropic layers, this reduces to one dimension, and 2-by-2 scattering matrices) [30]. As usual for unpolarized incident light, we average the transverse-electric and transverse-magnetic reflectances. The agreement is very good, except for the fact that the three mirrors' contributions appear somewhat narrow: slight random deviations from the ideal layer thicknesses, however, could easily account for this minor discrepancy.

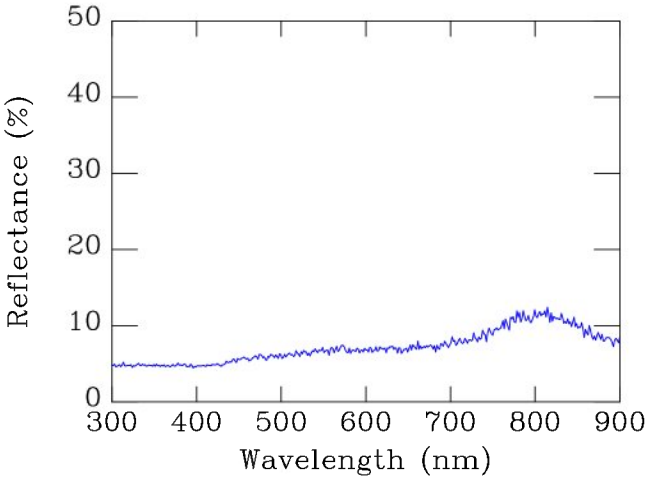

FIG. 12: (Colour online) Numerical simulation which describes the reflectance of the chirped photonic-crystal film described in Fig. 11, perturbed by random inhomogeneities. When the refractive index of the layer's porous part is allowed to fluctuate, the Bragg mirror is destroyed, and what remains is the effect of the pigmentary absorption. The incident light is unpolarized.

When the porous patches dry, their refractive index lowers and we see the formation of an unorganized distribution of extended scatterers that appear in the multilayer. This can actually destroy the Bragg mirror coherence and transform the optical reflecting layer into a translucent filter that gives a diffuse view on the red-pigmented underlayers. The material that makes up the rigid bulk of the elytron is indeed red, as can be seen when looking at the interior side of the elytron after mechanically removing the optically active layer. An indication for the reversible destruction of the Bragg mirror in presence of irregular dry porosity patches is provided by the model calculation shown in Fig. 12. In this model, the structure that provides (when perfect) the spectral reflectance in Fig. 11 is "corrupted" by random fluctuations of the reflective index, in the layers that contain the porous patch. In a given configuration, the refractive index of each layer is made to fluctuate between a maximum of 1.56 (the highest possible index of bulk chitin) and a minimum of 1.4, assuming, from inspection of the TEM images, that the volume occupied by the pores (darker areas in Fig. 7b) does not exceed 25%. The reflectance is averaged over a large number of random configurations (about 500, enough to stabilize the resulting reflectance spectrum). No correlation was introduced between successive layers, though this could be considered as a possible refinement of the model. As Fig. 12 shows, the bright "gold" reflection is destroyed, leaving only the reddish hue related to the pigmentary absorption of the substrate, included in the model.

VII. CONCLUSION

From the perspective of biology, the presence of such a complex structure on the external part of the insect's cuticle still raises many questions. A metallic gold aspect is often seen as an elaborate way of providing camouflage, as the (green) environment is efficiently reflected on this surface, making the insect very difficult to perceive (see, for instance the case of *Chrysina* (=*Plusiotis*) *resplendens*, a static gold insect from Costa Rica, that was already – but with an erroneous interpretaton – considered by A. Michelson in a 1911 paper [33]). However, it is not rare to see *Charidotella egregia*, sitting in the sun on a green leaf, displaying at certain times of the day. The gold colouration is quite generally the state in which the insect is found when mating. It is plausible (but not rigorously supported by biological evidence) that the metallic gold appearance is, in fact, an elaborate visual appearance, very conspicuous in full sunlight, when the insect seeks to increase the probability of being perceived by a mating partner. Also, the reflection

of sunlight by the metallic-gold surface probably limits the heat intake, providing another advantage, for an insect that cannot regulate this intake by reorienting the exposed surfaces (as butterflies do).

The biological role of the transformation into a "red" state is even more difficult to assess because it would require a long observation of the insect in the field. There are a large number of documented examples of very conspicuous insects which are unpalatable and red is a common colour for such insects, so the red colour may be dissuasive to predators in the insect's habitat. Another possibility relates to the observation that a missed attempt for predation often leads to the insect falling to the ground, where it starts the "red" transformation. A predator will probably continue to seek for a "gold-looking" prey and, as the transformation slowly progresses, *Charidotella egregia* acquires a better chance to escape. All these possibilities seem reasonable but none, to our knowledge, has been firmly verified by biological observation nor experimentation.

From the physics and material science points of view, this natural structure seems to be very interesting and new : we have a surface which becomes a "metallic" reflector when it acquires moisture. We can refer to this behaviour as "hygrochrome", underlining the change of colour with varying hygrometry. Tunable materials like electrochrome films (that change colour with varying applied electric fields) or thermochrome films (that change colour with varying temperatures) all have a strong potential for applications in sensing or switching devices. The new class of material evolved in *Charidotella egregia* could also find a number of uses in developing optical vapour sensors [34] or optical liquid gauges. Such optical sensors might be particularly appropriate, for instance, in detonating environments, when the use of classic electric probes would be too hazardous.

Acknowledgments

This work was initiated and largely carried out during the stay of two of us (J.P.V. and J.M.P.) at the Smithsonian Tropical Research Institute (Panama). J.P.V. acknowledges a travel grant from the Belgian National Fund for Scientific Research (FNRS). The study was also partly supported by the EU through FP6 BIOPHOT (NEST/Pathfinder) 012915 project and by the European Regional Development Fund (ERDF) and the Walloon Regional Government under the "PREMIO" INTERREG IIIa project. The authors acknowledge the use of Namur Interuniversity Scientific Computing Facility (Namur-ISCF), a common project between the FNRS and the University of Namur (Belgium). J.P.V. and J.M.P. acknowledge the Smithsonian Institution for providing facilities in Panama. M.R. was supported by the Belgian Fund for Industrial and Agronomic Research (FRIA). V.L. was supported as Research Associate by the Belgian FNRS. The authors thank Prof. Amand Lucas for many discussions and suggestions.

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