



Light manipulation by Christmas beetles:

quantification, mechanisms and ecological
relevance

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In celebration of all the non-eureka moments

To my cousins

Abstract

The brilliant and colourful appearances of Christmas beetles (Scarabeidae - Rutelinae) are famous for decorating eucalyptus trees during the Australian summer. Millions of years of evolution perfected the design of their hardened fore wings (elytra) to manipulate light and create striking optical effects. However, little is known about the exact underlying nanostructures or the ecological variables driving their evolution. Despite the recent increase in biophotonics studies, this remains the case for many organisms with striking colourful appearances. By studying light manipulation in Christmas beetles, I aim to develop methods and concepts generalisable to other organisms.

Christmas beetles produce a wide variety of optical effects including saturated colours, black, and pearlescent white. Some species look mirror-like and metallic gold or brass. Moreover, these colours change with the viewing or illumination angle. Christmas beetles can also interact with long wavelengths outside of the human-visible spectrum, in the near-infrared (NIR), and reflect circularly polarised light, which makes them appear as different colours through the right and left lenses of 3D cinema glasses. To better understand this diversity, I proposed a method for its quantification, studied some of its underlying mechanisms and tested if it can be explained by ecological differences.

To characterise the beetles' optical effects, I proposed a generalization of existing spectroscopy methods and parameters to describe reflection profiles across the visible and NIR spectrum, regardless of their underlying mechanism. Ultimately these methods break down complex optical effects into simpler traits and can facilitate comparison between studies. The proposed terminology attempts to conceptually unify disparate fields (biology and optics) and allows a clear distinction with terms used to describe colour perception.

To study the optical mechanisms in Christmas beetles, I analysed the architecture of their elytra. I discovered that unlike scarab beetles studied to date, three different Christmas beetle species use multicomponent photonic systems, with an upper layer acting as a green pigment-based filter and an underlaying broadband reflector, that particularly enhances NIR reflectance. For beetles with spiral nanostructures, I found a trade-off between polarisation and NIR reflectance. This diversity of photonic

structures shows that beetles are a promising model for understanding complex optical properties of natural materials.

To investigate ecological correlates of the optical effects in Christmas beetles, I used a biophysical essay and a phylogenetic comparative analysis. High reflectance reduced heating of the beetle elytra under controlled conditions, but I did not find any evidence that highly reflective species occur in hotter environments. Christmas beetles do not follow a simple eco-geographical pattern, possibly because their optical effects respond to species-specific combinations of environmental challenges.

I demonstrated that diversity in optical effects and photonic mechanisms is more than meets the eye, even for a small group such as Christmas beetles. My thesis highlights the value of a cross-disciplinary approach where optical methods can spark stimulating biological questions and the comparative study of phylogenetically related species can inform species selection for photonics studies. The resulting conversation between the two disciplines accelerates progress in the search for the biological function of striking optical effects.

Declaration

This is to certify that:

- This thesis comprises only my original work towards the PhD except where indicated in the *Preface*
- Due acknowledgement has been made in text to all other material used
- The thesis is fewer than 63,000 words in length, including tables, figures, references and appendices.

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Preface

I was the primary contributor to all sections of this thesis. I conducted the experiments and statistical analysis and led the manuscript preparation. All chapters were designed, produced and written with the kind support and guidance of my supervisors Devi Stuart-Fox, Ann Roberts and Allison van de Meene.

This thesis is presented as four main manuscripts. *Chapter 2* has been published as Laura Ospina-Rozo, Ann Roberts, Devi Stuart-Fox, 2022. [A generalized approach to characterize optical properties of natural objects](#), *Biological Journal of the Linnean Society* 137: 534-555. *Chapter 4* has been published as: Laura Ospina-Rozo, Jegadesan Subbiah, Ainsley Seago, Devi Stuart-Fox, 2022. [Pretty Cool Beetles: Can Manipulation of Visible and Near-Infrared Sunlight Prevent Overheating?](#), *Integrative Organismal Biology*, obac036, <https://doi.org/10.1093/iob/obac036>. *Chapter 3* has been revised and resubmitted following peer review in *Materials Today Advances*. *Chapter 5* is in preparation for publication.

Rather than including an introductory and concluding chapter, I have presented an integrative analysis in *Chapter 1*, where I combine the theoretical framework, knowledge gaps, key findings, and outstanding questions. I briefly reflect on the rationale behind the design of my thesis chapters and the challenges I faced, hoping that the lessons I learnt become useful for other researchers at the interface between optics and biology.

My PhD taught me to appreciate the benefits of producing shareable code for authors and readers, thus a link and QR code to readable, interactive web pages of my statistical analyses and data are provided at the end of each chapter.

For *Chapter 2 and 5*, Allison van de Meene and Vicki Bennett-Wood conducted the sample sectioning for TEM while I assisted with the sample preparation and imaging. In *Chapter 4*, I received technical guidance and training from Jegadesan Subbiah and Ainsley Seago, as well as their valuable comments on the final manuscript. For *Chapter 5*, Andrew Hugall assembled the phylogenetic tree and Katrina Rankin extracted RGB values from the calibrated photographs. Further details on collaborators' roles are described in a CRediT author statement section. Sources of funding and additional contributions are in an acknowledgements section at the end of each chapter.

All the photographs of Christmas beetles in this thesis were taken by me from museum specimens. The elements in their surroundings were artificially added for artistic purposes and do not represent their natural habitats. Digital art for the cover of each chapter was ideated by me and done by Francesco Battaglini.

Acknowledgements

To my old friends, to my new friends, a resounding thanks! for helping me grow so much.

This thesis would not have been possible without the guidance, support and kindness of my supervisors, Professor Devi Stuart-Fox, Professor Ann Roberts and Dr Allison van de Meene. At the beginning of this project, I had so many new concepts to learn, new techniques to try and new soft skills to develop. Along all the way, my supervisors helped me and gifted me with their time and wisdom. However, I do not think, for a moment, that they aimed to *only* pass knowledge into my brain. Beyond the invaluable training, they also created for me a safe environment to discuss the wildest ideas, to contemplate the “long shots”, to share the silly moments and frustrations. I believe that this kaleidoscope of shared discussions, dreams and emotions heavily shaped the final outcomes. I feel fortunate to have such powerful female role models as inspiration to continue my career, and I feel blessed for all the lessons they taught me, from scientific concepts to everyday life advice, because they have made me a better scientist and a better person.

To the members of my advisory committee, Iliana Medina and Mark Elgar, I owe a very special thank you. I am grateful for your valuable advice and unconditional support, as well as for the loving and honest words you gave me when I felt overwhelmed by the very real PhD monsters: impostor syndrome and lack of motivation. I treasure your words as powerful shields that give me strength to continue.

My thesis was supported by funding from the Australian Research Council (DP190102203, FT180100216) and I was able to complete my candidature with a scholarship from the University of Melbourne. In my project I used museum samples provided by the Australian National Insect Collection. I thank to the beetle division for their strong interest in encouraging research. In addition, during my first year, many people sent or collected beetles for me, especially Ainsley, Beau, Marty and Kat. So very much appreciated.

To my collaborators for all the things you have taught me and opening new opportunities for me. Specially to James Hutchison for your training and dedication. To my friends at the nanophotonics lab for the illuminating discussions, particularly Faris Shahidan for voluntarily reading papers on otherworldly animal photonics and taking the time to discuss them with me.

To my old friends, “the 2009 crowd” from my undergrad in Colombia: despite being scattered all around the world you were always ready for zoom drinks and PhD experiences forums. Thank you for letting our friendship grow stronger in the distance.

I am amazed of how many wonderful people I met during my PhD. They opened my eyes to new realities, enriching my science, my thoughts and my life. Though I won't be able to mention everyone here, I want to dedicate this paragraph to all those moments outside the lab, that make the magic inside the lab possible. Particularly, I want to say thanks to Katrina Rankin for the laughs and the “irregular language” that I always find so comforting. To Amanda Franklin for always being there when I panic before sending an email, or a manuscript, or a tweet. To Bryony Margetts for our special kind of feathery friendship. To Laura Cook for being so unconditional and for making me believe in myself. To Teruhito Ishihara for being such an amazing co-pilot, company in the adversity and a real friend from the heart since time = 0. To Magdalini, my girl-friend, who taught me the real meaning of being a woman-friend, the love, the kindness, the wonders it entails. To Haydn, for his quantum non-theoretical-instead-very-real love. To the L's gang: LuYi Wang and Leslie Ng, thank you for teaching me patience and courage, and for just generally making my days brighter.

I feel very lucky and honoured to call past and present members of Stuart-Fox Lab and Medina Lab, my friends. I deeply thank you for creating and nurturing such a great culture, and as Caroline once said, “for reminding me that good science does not have to be stiff, boring and competitive”. With you as lab mates, science is categorically the opposite to that.

I want to thank my family for believing in me even when I doubt, for reading my abstracts, connecting to my online talks and liking my tweets, in summary, for making me feel so loved. To my parents for their love, patience, and bravery to face the uncertainty of the future and the butterfly effect delirium of the past. Thank you for doing everything in your power to enable me to follow my passion and curiosity, even from the other side of the world. Thank you for always being at one-text-message distance and ready to cheer me up. Finally, to my amazing partner Coco, I am grateful because you valued and respected my creation so much that you invested your time, knowledge, and creativity to see me reaching the finish line... and for all the tea...

Finally, I thank you, the reader of my thesis, for your time and for giving life to these words that took so long to finally see the light.

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Chapter 1 - Introduction



"We can only love what we know, and we can never know completely what we do not love. Love is a mode of knowledge"
Aldous Huxley

1.1 | Light waves and Living organisms

Colour produced by living organisms has fascinated humans since ancient times. It can be bright, conspicuous and sometimes changeable, properties that are extremely difficult to recreate with our pigments and paintings (Schenk *et al.*, 2013). It is fascinating how some natural colours can resist the passage of time. Fossils millions of years old have been found with their colours intact, or at least the structures are sufficiently conserved that the original colours can be reconstructed (McNamara *et al.*, 2012; McNamara & Orr, 2013; Carney *et al.*, 2012; Zhang *et al.*, 2010). For biologists, colours and colour patterns are a functionally important and measurable component of the phenotype, at the interface between an organism and its environment, thus a model to study ecology and evolution (Endler & Mappes, 2017). For physicists, engineers and artists, natural colours, particularly those involving nanostructures, have become a source of inspiration for the development of a wide range of technologies such as cameras, sensors, smart clothing and even iridescent chocolate (Srinivasarao, 1999; Braun, 2011; Caro *et al.*, 2017; Schroeder *et al.*, 2018). And for animals, colour can be quite literally, a matter of life or death. Colour, but more accurately, the interactions between electromagnetic waves and living organisms, can be a communication channel between social partners, mates, predator and prey, and they can mediate relationships with the environment affecting the organism's temperature and water balance amongst others (Doucet & Meadows, 2009; Endler & Mappes, 2017; Stuart-Fox & Moussalli, 2009; Johnsen, 2012). These interactions with light are so important that they can drive rapid evolution at large scales (Andrew Richard, 2000).

During my PhD I fell in love with colour science. I enjoyed talking about my research with people from all backgrounds and interests, because almost invariably, powerful emotions surfaced in those conversations. At times just a hint of curiosity, at others even fascination for the colours of nature. I think there is beauty in realising that organisms distant from humans and sometimes disregarded as “simple” or “ugly” (often the case for insects) have very sophisticated ways to interact with electromagnetic waves. Noticing that what we perceive as “everyday colour” is the product of very cool optics tricks perfected by evolution, can make us humans feel closer, more interested in knowing and preserving the lives of these fantastic beasts. I think this is a powerful reason to study how and why organisms filter, reflect, absorb, twist or slow down - in fewer words - manipulate light.

Table 1-1. Glossary for some of the technical terms used in this thesis.

The definitions are based on the concepts in Johnsen (2012) unless indicated.

Term	Definition
Scattering	When one photon interacts with the atoms and molecules of an object, a new photon is emitted making it appear as if the light bounces - not necessarily in the original angle. This is considered a non-absorbing/elastic interaction because the incoming and emitted photons have the same energy, thus the same wavelength.
Constructive interference - Coherent scattering	When two waves of the same wavelength and phase interact, they add together so that the amplitude of the resultant wave is the sum of the two amplitudes of the original waves.
Destructive interference	When two waves of the same wavelength and a difference in phase of 180° interact, they cancel each other out.
Incoherent scattering	Scattered waves have random relative phases
Iridescence	A change in wavelength with viewing or illumination angle (Stuart-Fox <i>et al.</i> , 2020; Meadows <i>et al.</i> , 2009; Starkey & Vukusic, 2013)
Specularity	A change in the amount of light reflected with viewing or illumination angle (Stuart-Fox <i>et al.</i> , 2020; Gruson <i>et al.</i> , 2019a)
Specular angle	An angle of the same magnitude as the angle of incident light, but opposite direction
Refractive index (RI)	A number describing how fast light travels through a given material. It is the ratio of the speed of light in a vacuum to the velocity of light within a material ($RI = c/v$), and it is a complex number (with a real and an imaginary part) For biological materials, this value is the square root of the permittivity (this concept is more common in photonic studies), and its imaginary part relates to the absorption of the material. Importantly, the RI is wavelength dependent, and it does not correlate directly with the density of a material.
Photonic crystals	A repetitive structure that produces scattering through interference. If the index of the refraction varies only in one dimension, the structure is called a 1D photonic crystal. There are also 2D and 3D photonic crystals. Some examples of 1D photonic crystals are thin films, Bragg Stacks (multilayers) and diffraction gratings. Opals are an example of a 3D photonic crystal.

1.1.1 Diversity of optical effects in nature

In many studies the expression “structural colour” is used to describe the wavelength selective **scattering** that arises from the interaction between light and nanostructure, in contrast to colours produced primarily by chemical absorption of light by pigments (Starkey & Vukusic, 2013). The term ‘structural colour’ is problematic because the word “colour” is strongly associated with perception, referring to reflection of light waves in the human-visible (400 to 700 nm) or, more broadly, the animal-visible spectrum (300 - 700 nm). However, the full spectral range of sunlight (~280-2600 nm) also includes near infrared wavelengths (NIR, 700 to 2000 nm) not necessarily used for visual communication (Nussear *et al.*, 2000; Luke, 1994; Stuart-Fox *et al.*, 2017; Clusella Trullas *et al.*, 2007). In addition, the word “structural” is mainly used to indicate reflection that is produced only by nanostructures through **constructive interference** (Shawkey *et al.*, 2009a; Starkey & Vukusic, 2013). But in many situations the underlying mechanism is uncertain and cases where pigments and structures interact are more the rule than the exception (Shawkey & D’Alba, 2017). Finally, the study of structural colour is biased towards analysing wavelengths of light reflected by a surface. For natural structures, not only the wavelength is relevant, but also the proportion of total light reflected (Kemp *et al.*, 2015), and the angle in which these wavelengths are reflected (Gruson *et al.*, 2019a; Zobl *et al.*, 2020). For these reasons, in this thesis I use the broader expressions light manipulation or optical effects to encompass the way in which light interacts with an organism’s surface across the full wavelength range of sunlight.

Optical effects produced by natural materials are described in the literature using a multitude of terms: flashy, shimmering, glossy, shiny, brilliant, iridescent, metallic, opal, mother-of-pearl, etc (Stavenga, 2021e.g., ; Mouchet & Vukusic, 2018; Waldron *et al.*, 2017; Iskandar *et al.*, 2016; Wilts *et al.*, 2013; Maia *et al.*, 2011; Schultz & Fincke, 2009; Meadows *et al.*, 2009; Han *et al.*, 2008; Thomas *et al.*, 2007; Huxley, 1975). The heterogeneity in terminology responds to the need to describe the amazing variety of optical effects that animals can produce. But sometimes it can prevent comparison between studies and a clear notion of the properties measured in each case. For example, structural colours are often divided into **iridescent** and non-iridescent (Osorio & Ham, 2002; Shawkey *et al.*, 2009a), but there are some cases when the change in angle causes a more significant change in intensity, which is technically a different property, termed **specularity** (Stuart-Fox *et al.*, 2020; Wilts *et al.*, 2013). Describing and characterising the enormous diversity of light manipulation in the natural world remains an enduring challenge and is a focus of this thesis.

One way to tackle the task of characterising the diversity and complexity of light manipulation is to distil a set of simpler properties that can be measured in a consistent and repeatable way. Although still challenging, it is possible to name and define these properties or traits and then use them to describe complex effects. This was the inspiration for the objectives in Chapter 2 of this thesis. In addition, I had the opportunity to contribute to an opinion piece on the concept of iridescence and a quick guide for gloss (Stuart-Fox *et al.*, 2020; Franklin & Ospina-Rozo, 2021).

1.1.2 Light manipulation mechanisms

The diversity of optical effects in nature extends to their underlying mechanisms (some examples in **Figure 1-1**) (Fox & Vevers, 1960; Srinivasarao, 1999; Vukusic & Sambles, 2003; Prum *et al.*, 2006; Fox, 1976; Bagnara *et al.*, 2007; Mathger *et al.*, 2009; Kinoshita *et al.*, 2008; Seago *et al.*, 2009; Shawkey *et al.*, 2009a). Such variety may appear overwhelming and difficult to study, but a common approach has been to classify optical effects into categories such as pigment-based, structural, iridescent, non-iridescent, etc. (Fox & Vevers, 1960; Hill *et al.*, 2006; Shawkey & Hill, 2005). However, the boundaries between these categories are sometimes blurred. Instead, the following two general principles apply to any light manipulation mechanism:

All optical effects arise due to a change in the path of the light, i.e. scattering. Although every single molecule in a material causes scattering, current optical equations are based only in sudden, more significant changes in the path, which are produced when the light beam encounters a big change in **refractive index**. As a result, the refractive index of each material, the size, and separation of nanostructures determine the effects we see at the macro scale. *“...scattering [is] a simple phenomenon that is computationally messy, rather than a set of different phenomena”* (Johnsen, 2012).

Modularity, i.e. the combination of elements at different hierarchical levels can create complex optical effects. This may refer to the combination of two stacks of structures optimized to produce different effects (Kinoshita *et al.*, 2008; Kinoshita & Yoshioka, 2005), or to the combined layers of structures and pigments since *“Without scattering, all pigmented substances are black [~transparent] when viewed via reflected light”*. (Johnsen, 2012; Shawkey & Hill, 2005).

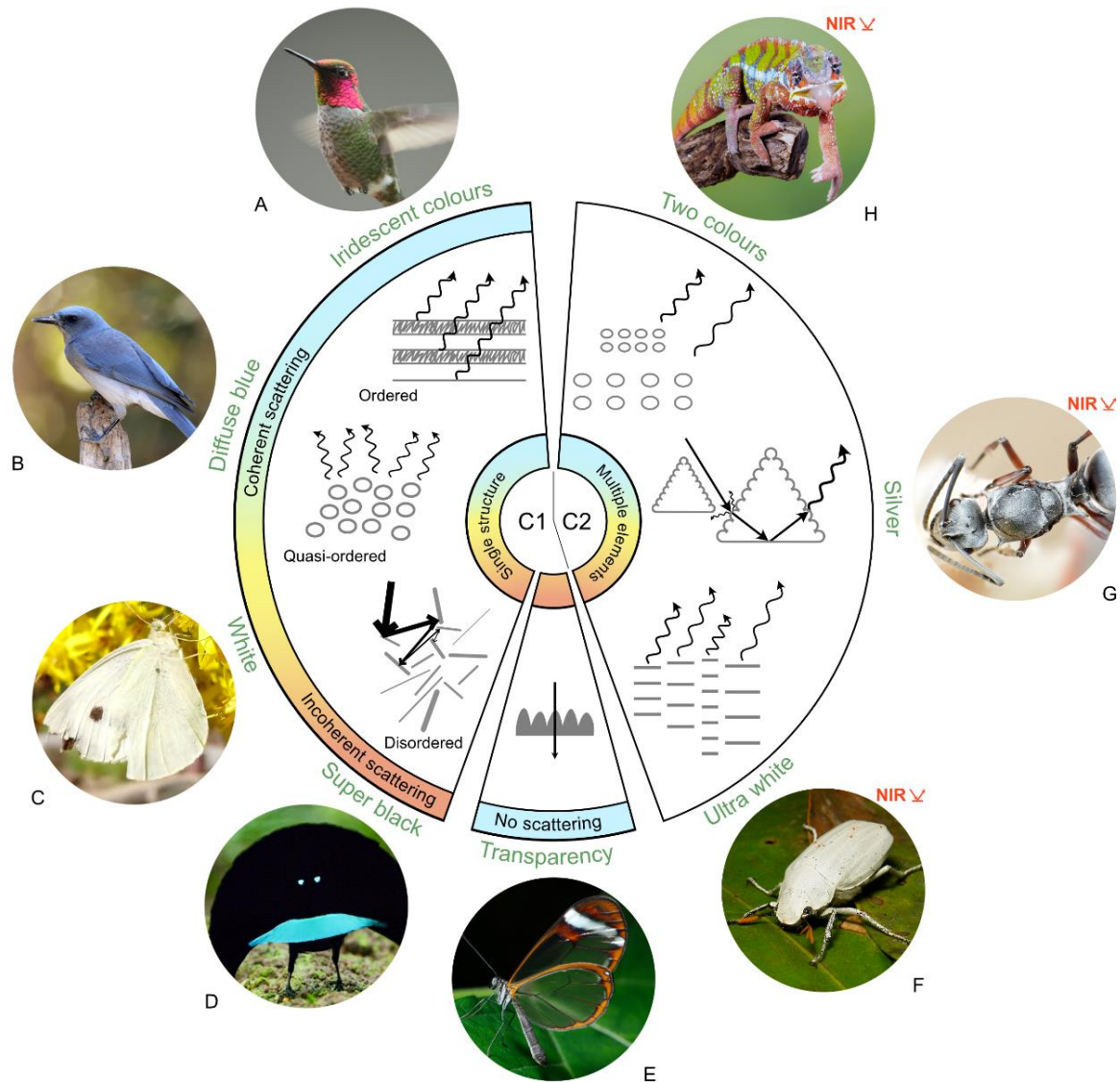


Figure 1-1. Examples of light manipulation mechanisms

A. *Calypte anna* (Giraldo *et al.*, 2018) - B. *Aphelocoma wollweberi* (Prum *et al.*, 1999a; Vinther, 2020) - C. *Pieris rapae* (Stavenga *et al.*, 2004) - D. *Lophorina superba* (McCoy *et al.*, 2018) - E. *Greta oto* (Siddique *et al.*, 2015) - F. *Cyphochilus sp.* (Hallam *et al.*, 2009) - G. *Cataglyphis bombycina* (Shi *et al.*, 2015) - H. *Furcifer pardalis* (Teyssier *et al.*, 2015). Photos reproduced under Creative Commons Attribution 2.0 Generic license, authors are: A-B. Alan Vernon, C. Wikipedia user Spisah, D. Tim Laman, E. Magnus Hagdorn, F. John Horstman, G. Albert Tamayo, H. Sue Cro.

Considering that all forms of scattering are changes in the path of light, **incoherent** and **coherent scattering** are not two different phenomena but rather part of a continuum. Coherent scattering (constructive interference) is produced by ordered nanostructures with size in the range of approximately $\frac{1}{4}$ wavelength that reflect specific wavelengths aligned in phase, mainly at the **specular angle**, generating a saturated iridescent colour. This is the case for thin films, Bragg stacks in **Figure 1-1A**, diffraction gratings and 2D or 3D **photonic crystals** (Prum *et al.*, 2006; Kinoshita *et al.*, 2008; Srinivasarao, 1999; Stavenga, 2014; Starkey & Vukusic, 2013). In contrast,

incoherent scattering is produced by disordered arrays of relatively large particles ($>> \frac{1}{4}$ wavelength) that reflect a wide range of (non-aligned) wavelengths at different angles generating a diffuse desaturated appearance (e.g., Mie scattering in Fig 1C). Intermediate effects can be achieved by adding disorder in the size and spacing of the nanostructures (e.g., quasi-ordered arrays in **Figure 1-1B**) (Bermudez-Urena *et al.*, 2020; Shawkey *et al.*, 2009b). However, not all types of irregularities cause the same effect; for example, a gradual change in size and spacing can produce enhanced broadband specular reflection (gold/silver), while offsets in the structures are likely to create diffuse broadband reflection (white, Fig 1F). The role of disorder has been largely reviewed in recent studies (Kinoshita & Yoshioka, 2005; Poladian *et al.*, 2009; Shawkey *et al.*, 2009a; Vukusic & Stavenga, 2009)

Light manipulation with nanostructures is not limited to structural colour. Transparency (**Figure 1-1H**) can be achieved by reducing scattering to the minimum. Light doesn't change its path if it does not encounter a boundary between two media with different refractive indices (for example air and tissue). Conical nanostructures smaller than most light waves (max ≈ 250 nm) incorporate disorder in their position and height (Siddique *et al.*, 2015) to create a transition layer between the two indices of refraction so the light can pass through (Schroeder *et al.*, 2018). The opposite effect, structural absorption (present in some whites and in super black - **Figure 1-1A**), can be achieved by increasing incoherent scattering. Every time light gets reflected, part of it is transmitted and absorbed by the material. Densely packed disordered arrays increase the number of boundaries between indices of refraction (for example between air and chitin or keratin), thus forcing the light to “bounce” inside the structure until the absorption by the material becomes significant (McCoy *et al.*, 2018; Vukusic *et al.*, 2004). Transparency and structural absorption are the perfect example of how different types of disorder and particle scales can create widely different effects. As a result, the role of disorder in natural light manipulation systems has become a central focus of photonic studies in recent years (Bermudez-Urena *et al.*, 2020; Johansen *et al.*, 2017; Rothhammer *et al.*, 2021).

Modularity explains how it is possible to combine different optical properties into complex effects (Kemp, 2002; Wilts *et al.*, 2013; Shawkey & D'Alba, 2017; Choi & Byun, 2019). Although modularity can create complex mixed effects in the visible range of the spectrum, here I want to draw attention to how this combination of various elements can be critical for near infrared reflection. With only one structure, high NIR reflectance is strongly correlated with reflectance of longer visible wavelengths (red),

or in some cases purple (Magkiriadou *et al.*, 2014). NIR is also commonly associated with broadband reflectance (Hallam *et al.*, 2009; Stuart-Fox *et al.*, 2018), which could be because repetitions of one structure at different scales (**Figure 1-1G and H**) are relatively easy to achieve. Another strategy to enhance NIR reflection is to have one structure with two hierarchical levels, one manipulating NIR and another one visible light (**Figure 1-1G**). Finally, a powerful strategy to reduce the correlation between visible and NIR reflectance is to have two separate layers of structures (**Figure 1-1F**), and this could mean they evolve in response to different selective pressures.

Multiple studies have shown the importance of considering the interactions between pigments and structures (Grether *et al.*, 2004; Vukusic *et al.*, 2004). Pigments and other molecules can be embedded in the structures changing the refractive index of the material (Caveney, 1971), or they can interact synergistically with the structures (Shawkey & D'Alba, 2017; Shawkey *et al.*, 2009a; Morehouse *et al.*, 2007; Shawkey & Hill, 2005; Yoshioka & Kinoshita, 2005; Prum & Torres, 2003a). Some of the advantages of combining layers of pigment and structures will be further discussed in *Chapter 3*.

The two general concepts of changes in the path of light and modularity of structures can explain why there are so many ways to achieve coherent and incoherent scattering across the full spectrum (visible and NIR) starting from simple building blocks. However, comparing organisms that are phylogenetically distant (**Figure 1-1**) offers little information about how “easy” or “difficult” it is to modify the basic structures to obtain different effects, or the advantages or trade-offs associated with each structural configuration. More studies comparing closely related species are needed (but see Gomez *et al.*, 2021; Gruson *et al.*, 2019b). A major goal of this thesis was to take phylogenetically informed comparative approach to understanding the diversity of light manipulation by focusing on optically diverse Christmas beetles.

1.1.3 Function and evolution

The dazzling optical effects found in nature are so diverse, it is no wonder they have attracted extensive research on their function and evolution. Due to the striking appearances of structural colours and their dynamic character, it is natural to think they must have a role in communication or predator avoidance (reviewed in Doucet & Meadows, 2009; Andrew Richard, 2000; Seago *et al.*, 2009; Ghiradella, 1999; Gronquist *et al.*, 2006). Counterintuitively, iridescence has been shown to also play a role in camouflage (Kjernsmo *et al.*, 2018; Kjernsmo *et al.*, 2022; Kjernsmo *et al.*, 2020). The

literature on biophotonics and bioinspired materials often assumes a communication or camouflage function; however, this assumption ignores the ecological context, traits that influence the appearance of natural objects to different receivers and potential non-visual functions. The appearance of natural objects depends on many factors, including the visual capabilities of different receivers (e.g. conspecifics, predators, prey) which differ greatly among species, illumination conditions and the ability to control the display of the optical effect (Kemp *et al.*, 2015; Cuthill *et al.*, 2017; Endler, 1990; Endler, 1993; Endler & Mappes, 2017; Osorio & Vorobyev, 2008). In addition, light manipulation may have other functions such as including vision enhancement (tapetums), thermoregulation, water repellence, mechanical resistance, friction reduction, photoprotection, or self-assembly (Schroeder *et al.*, 2018; Eliason & Shawkey, 2011; Doucet & Meadows, 2009; Mendes *et al.*, 2013). In some cases, photonic structures could be simultaneously selected for several of these functions, while in some others, amazing optical effects could arise merely as a by-product of selection for one of the aforementioned functions. Disentangling and testing all these scenarios is a demanding task.

The challenge of understanding the function of biophotonic structures is accentuated by the fact that the choice of research question and study organisms often arises in a rather serendipitous way (Schroder-Turk, 2020). A biologically informed choice of organisms could offer a more direct path to finding a link between form and function. Overall, the fields of optics and material sciences have excelled in describing and characterising the material properties of elaborate natural photonic systems, but they find themselves in need of a deeper connection with biology to understand function (Schroder-Turk, 2020). In this thesis, I discuss two strategies to address the potential selective pressures driving the evolution of the optical properties in biological materials.

One strategy is to design experiments that focus on the biological role of specific optical properties, such as iridescence, and distinguish their effect from other properties with which they are often confounded (McClure *et al.*, 2019; Arias *et al.*, 2019). During my PhD I had the opportunity to collaborate on exciting projects aiming to do precisely that. For example, one hypothesis is that bright colourful surfaces are a by-product of the regular nanostructures required to enhance hydrophobicity (Gans & Baic, 1977; Gower, 2003; Wagner *et al.*, 1996; Barthlott & Neinhuis, 1997). We tested if iridescence correlates with hydrophobicity for six species of plants, but we did not find evidence for such correlation because different structures with varying optical

properties can produce similar levels of hydrophobicity (Garcia *et al.*, 2020). In another example, a mirror-like appearance has been proposed to be a camouflage strategy since it allows to blend in by reflecting the surroundings (Denton, 1971). We tested whether high specularity reduced predation of beetle clay models, but we did not find evidence for this hypothesis (Franklin *et al.*, 2021). In a third study, we isolated the spatial and temporal component of iridescence to test which of them can be perceived by honey bees. Since bees could learn the dynamic nature of iridescence (the temporal changes in wavelength), we concluded that animals may have the neural mechanisms to pay attention to changeable colours and could potentially use this information in ecologically relevant contexts (Ng *et al.*, 2022). Finally, I contributed to an opinion piece in which we argued that iridescent colour patches are only likely to be used as signals when accompanied by mechanisms to precisely control the angular change and corresponding appearance to the viewer (Stuart-Fox *et al.*, 2020).

Another strategy to elucidate function and evolution is to compare organisms considering their phylogenetic relationships. If a trait or mechanism is present in most members of a group (including the most basal species) it probably originated due to common ancestry. For example, 3D photonic crystals in weevils emerged as a camouflage strategy due to a change in diet of the ancestor (Seago *et al.*, 2019). Similarly, the transition between a hollow lumen to one bearing 1D or 3D photonic crystals in *Heliconius* butterflies has happened only once in their evolutionary history (Lloyd & Nadeau, 2021; Brien *et al.*, 2022). The opposite scenario is evolutionary convergence, when a trait appears independently multiple times in non-sister groups in response to a common selective pressure. An example is transparency in butterflies and moths to camouflage and thermoregulate (Gomez *et al.*, 2021), or high NIR reflectance for passive thermoregulation in birds and butterflies (Medina *et al.*, 2018; Munro *et al.*, 2019). Diffuse blue is a convergent trait in birds but its causes are still unclear (Prum & Torres, 2003a). Without considering the evolutionary history of organisms, it is impossible to distinguish common ancestry from convergent evolution in response to specific selection pressures. Thus, a comparative approach is a powerful tool to study the function of diverse optical effects.

1.2 | Beetles and Christmas Beetles

1.1.4 Beetles - Coleoptera

I imagine almost every book or thesis section about beetles starts by citing the possibly apocryphal, yet illustrative story of the British biologist J.B.S. Haldane, who, on being asked what one could conclude about the Creator from a study of their creation, answered that “God has an inordinate fondness for beetles.” This thesis is no exception. Considering that almost one quarter of all living creatures on earth (excluding bacteria and archaea) are beetles, it is clear they are the most successful animal (Stork, 2018; Grimaldi *et al.*, 2005). Coleoptera is also the most diverse of the insect orders (Grimaldi *et al.*, 2005) with approximately 350000 species described and 5-30 million estimated (Lawrence & Britton, 1994), eight times as many species as there are vertebrates (CSIRO. Division of Entomology & Naumann, 1991). Beetles occupy almost every non-marine habitat in the world being, in consequence, enormously diverse in appearance and size (Lawrence & Britton, 1994).

Key to the success of beetles is the modification of the first pair of wings into a “shield” that protects the hindwings called elytra (Fedrigo & Wray, 2010; Grimaldi *et al.*, 2005). The elytra enabled beetles to colonize a wide variety of niches without losing their ability to fly (Lawrence & Newton, 1982). This modification included the thickening of the veins in the forewings (Fedrigo & Wray, 2010; Tomoyasu *et al.*, 2009) followed by the development of interlocking mechanisms and co-adaptation with the abdomen and thorax to create the subelytral cavity (Lawrence & Newton, 1982). This cavity can have a role in thermoregulation, water retention (Linz *et al.*, 2016; Draney, 1993; Duncan, 2002; Schilman *et al.*, 2008) and respiration in aquatic environments (32 diving species Ponomarenko & Prokin, 2015). Thus, beetle elytra are a multifunctional masterpiece of nature.

Elytra are also very diverse in appearance (Seago *et al.*, 2009). That is because they are made of a versatile material called chitin, which can be organized in chiral fibres (Gunderson *et al.*, 1991) and/or form hierarchical complex organizations to interact with light (Van de Kamp & Hartmut, 2010). As a result, beetles can achieve iridescence, ultraviolet and NIR reflections, polarisation, iridescence, opal appearances and pointillistic colour mixing amongst others through variations of four main mechanisms: multilayer reflectors, 3D **photonic crystals**, diffraction gratings and disordered/quasi-ordered arrays (Seago *et al.*, 2009). Some species can even change colour reversibly

(e.g. Rassart *et al.*, 2008; Vigneron *et al.*, 2007). Although the list of precise optical mechanisms found in beetles is already long, there are many that remain to be fully explored, and potentially more to discover.

Identifying the functions of light manipulation is a challenging enterprise that entails imagination and creativity. Some of the functions that have been proposed for various optical effects include crypsis against a green background (Crowson, 2013; Parker *et al.*, 1998) or against the colour of small stones (Hadley *et al.*, 1988; Pearson & Vogler, 2001; Schultz, 1986; Knisley & Schultz, 1997), sexual signalling (Alcock, 1976) warning colouration (Shelly & Pearson, 1978; Pearson & Knisley, 1985; John, 1988; Vogler & Kelley, 1998), camouflage (Kjernsmo *et al.*, 2020; Kjernsmo *et al.*, 2022) and thermoregulation (Hadley *et al.*, 1992). Certain optical effects could also be a secondary result of other functions. Given that the ecology and habitats of different beetle species are so diverse and specialised, it is essential to consider the biology of the species when evaluating the potential function of light manipulation (Seago *et al.*, 2009). This includes the visual system of the study organism and/or its predators, behaviour and microhabitat preferences. Equally importantly, we must move beyond the traditional approach of *only* studying biophotonics of species chosen for their interesting appearance (e.g., selected from collections or purchased), in isolation from their ecological and evolutionary context.

1.1.5 Scarabs

The order Coleoptera is divided into 160 different families and Scarabaeidae is the second largest with over 27000 species worldwide (Cassis, 1992) from which 2000 are present in Australia (Lawrence & Britton, 1994). Beetles in this family have characteristic antennae with large clubs and lamellae that can be fanned out to detect chemical cues or closed to protect the sensory organs. Australian scarabs cannot roll their body into a ball and their head is clearly visible, but their mouth parts are hidden under the edge of the head, a structure called “clypeus” (Lawrence & Britton, 1994). Feeding habits determine major structural modifications in this group and separate two well supported lineages: phytophagous and saprophagous (Gunter *et al.*, 2016; Ritcher, 1958). Thus, the great diversification of scarabs has been correlated both to the diversification of angiosperms, and a change to a diet based on vertebrate dung (Gunter *et al.*, 2016; Halffter, 1991).

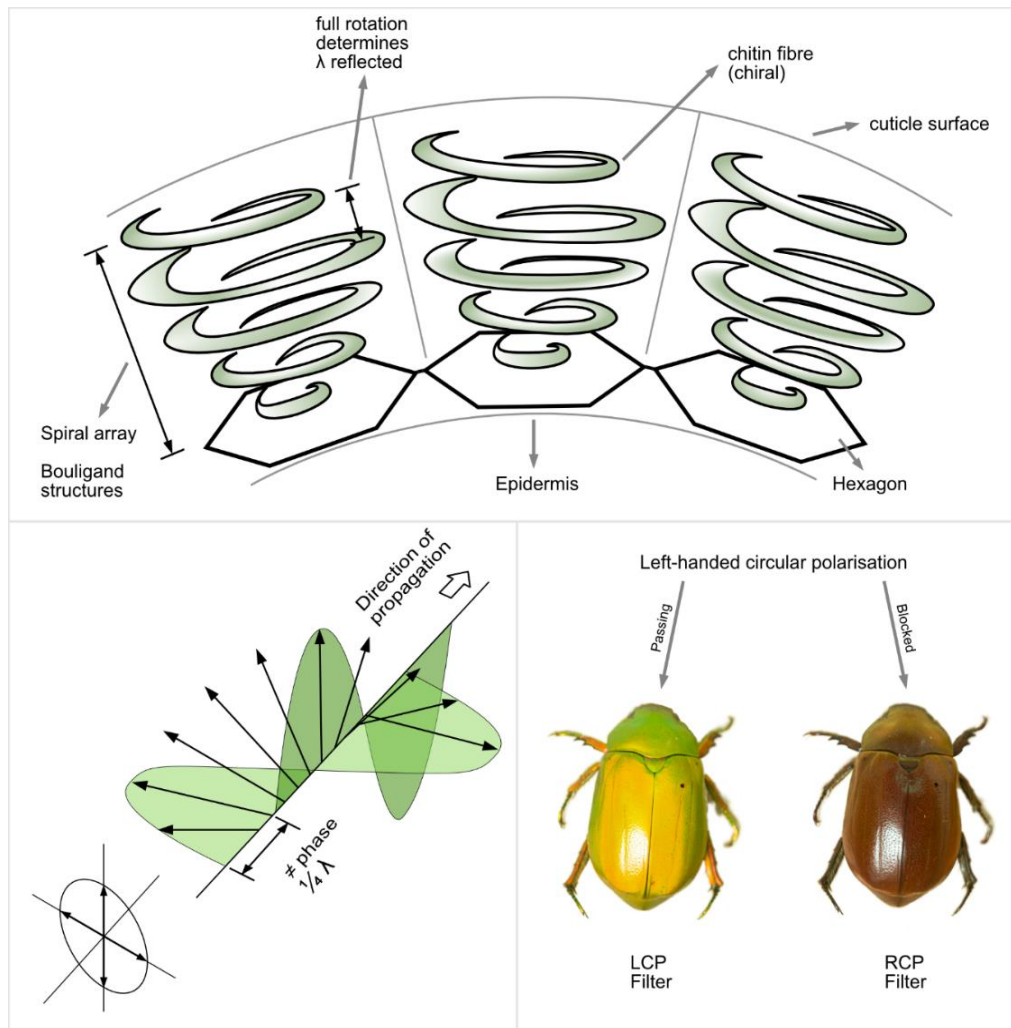


Figure 1-2. Scarabs reflect circularly polarised light

The Scarab cuticle contains fibres of chiral chitin packed in a spiral array. This array has optics analogous to cholesteric liquid crystals and selectively reflects left-handed circularly polarised light (Neville & Caveney, 1969; Arwin *et al.*, 2012; Hernández-Jiménez *et al.*, 2014; McDonald *et al.*, 2017; Mitov, 2017; Sharma *et al.*, 2014; Yang *et al.*, 2017). Circular polarisation is produced by interference of two waves of the same wavelength, perpendicular electric fields, and a difference in phase of $1/4\lambda$. The electric field of the resulting wave appears to rotate circularly (Johnsen, 2012). Since chiral nanostructures reflect only left-handed circularly polarised light (*C. resplendens* is the exception Finlayson *et al.*, 2017), their colours disappear under filters that impede the passing of this type of light. The wavelength of circularly polarised light may be detected by photoreceptors, but to the date, adaptations in the visual system to detect the circularity of the electric field have only been found in the mantis shrimp (Chiou *et al.*, 2008).

Scarabs are famous for reflecting circularly polarised light. In fact, circular polarisation by natural materials was first reported more than a century ago in a scarab by Michelson (1911), who attributed this effect to a microscopic “screw structure”. Since then, there has been abundant research on the optics and underlying mechanisms of circular polarisation (**Figure 1-2**) but its function remains unknown. Interestingly, scarabs have specialised photoreceptors to detect linear polarisation for navigation (Dacke *et al.*, 2002; Labhart *et al.*, 1992; Marshall *et al.*, 2019), but there is no evidence that they have sensors nor behavioural responses to circularly polarized light

(results in Brady & Cummings, 2010; refuted by Blaho *et al.*, 2012; Horváth *et al.*, 2014). Circular polarisation could be an indirect result of selection for enhanced mechanical resistance (Wu *et al.*, 2020), bright or metallic structural colours (Miao *et al.*, 2015) or self-assembly (Mitov, 2017; Burg & Parnel, 2018).

Studying circular polarisation in an evolutionary context is relevant and needed. Amongst beetles, circular polarisation is almost exclusively found in scarabs (except for one genus of Hybosoridae) (Michelson, 1911; Pye, 2010). Polarisation may be an ancestral feature in scarabs since it is present in 8 out of the 15 subfamilies (Pye, 2010), but it is unknown why it is absent in the others. In addition, species vary in their degree of polarisation (Bagge *et al.*, 2020; Goldstein, 2006; Pye, 2010). To the date, fossil examples of polarised beetles have not been reported, but it is unclear whether this means that circular polarisation appeared recently in the evolution of scarabs (Odin *et al.*, 2018). Even if circular polarisation is the result of selection for other properties, one useful approach to understand its evolution could be to explore what biotic or abiotic factors are common for polarised beetles and absent in non-polarised ones in a group of closely related species.

1.1.6 Christmas beetles

- Identification and Morphology

In Australia, the common name “Christmas beetle” is used to refer to sparkly colourful beetles that are active in summer, around Christmas time (**Figure 1-3**). Strictly speaking, it refers to the species that belong to the genus *Anoplognathus* comprising 35 species (Hangay & Zborowski, 2010; Lawrence & Britton, 1994). However, this common name has also been used loosely for other scarabs, melolontins and even lucanid beetles from the genus *Lamprima*. In this thesis and associated papers, we use the common name to refer to scarabs in the subfamily Rutelinae that occur in Australia, approximately 109 species and 23 genera (Cassis, 1992). We included two species from the genus *Xylonychus* subfamily Melolonthinae due to their similar locations and appearance (see the [Australian Museum website](#) for a user-friendly classification key).

The main characters to identify a Rutelinae scarab are their general shape, and the terminations of their legs (Hangay & Zborowski, 2010; Lawrence & Britton, 1994). Rutelinae scarabs never have prominences in their head or pronotum unlike related genera of the Rhinoceros beetles and Dung beetles. In Rutelinae, the terminal part of the body (pygidium) is visible beyond the end of the elytra, and at least one pair of the abdominal spiracles (respiratory organs) is exposed below the margin of the elytra. The tarsi in the second pair of legs are divided in 5 segments, and the claws in their hind legs are unequal, flattened and flexible. Some of the most common genera, and the ones considered in this thesis are *Anoplognathus*, *Anoplostethus*, *Calloodes*, *Repsimus*, *Anomala*, *Epichrysus*, *Mimadoretus*, *Anomala* and *Paraschizognathus* (Carne, 1958; species of *Anoplognathus* in Seidel & Reid, 2021; Carne, 1957).

In most genera of Christmas beetles there is no evident sexual dimorphism apart from the reproductive organs and very subtle differences that vary between species. For example, *Anoplognathus* females have a less squared and less emarginate clypeus than males. Two genera show very strong sexual dimorphism: *Paraschizognathus* males have much bigger eyes and antennae than females. In *Repsimus* males have big claw in their hind legs that they use to hang upside down from the leaf stalks while entirely carrying the female during copulation (Carne, 1958).



Figure 1-3. Christmas beetles

First row: *Anoplognathus smaragdinus*, *Anoplostethus laetus*, *Anoplognathus montanus*, Second row: *Calloodes grayanus* (top), *Anoplognathus aureus* (bottom), *Anoplostethus laetus*, *Anoplognathus prasinus*. Third row: *Anoplognathus smaragdinus*, *Anomala antiqua*, *Anoplognathus porosus* (Classification based on ANIC records and Christmas Beetles app.)

▪ Life cycle

Christmas beetles have a 1 to 2 year life cycle, most of which corresponds to the immature stages, since adults' life span can be roughly three months or as short as 15 days in some species of the *Calloodes* genus. Females lay the eggs during late summer. Each female can lay around 25 to 30 eggs compressing the soil around each egg (Carne, 1957), or around clusters of maximum 3 eggs in *Calloodes* (Carne, 1958). The larvae hatch the following year with the heavy rains of spring, but unfavourable conditions, particularly drought, may delay or prevent the hatching (Carne, 1957). Larvae of *Anoplognathus* sp. are found in the soil and feed on rotting wood, roots or crops (Carne, 1957). The larvae of *Anomala* sp. can be found in rain forests and red volcanic soils, while the larvae of *Repsimus* sp. can be found in sandy soils along riverbanks and larvae of *Calloodes* sp. can be found in pure sand (Carne, 1958). The food sources for the larvae in these last three genera are unknown. In general, adults emerge from the pupae at the beginning of summer and remain active for the season (Steinbauer & Weir, 2007; Carne, 1957). Curiously it has been reported that *Calloodes* adults can emerge in October, but they do not fly until February (Carne, 1958).

▪ Ecology

Most species of Christmas beetles occur near the coast while very few inhabit the arid or desertic regions of central Australia (Carne, 1958). Members of Schizognathina (not analysed in this thesis) have special adaptations to harsh environments, such as greatly modified sensory organs and reduced mouthparts in the males (Carne, 1958). Christmas beetles inhabit eucalyptus forests, but they may be found in parks of big cities, in backyards or close to crops (according to museum tags and Atlas of Living Australia). Data on the biology of Christmas beetles is usually limited to *Anoplognathus*. For other genera most of the information comes from field observations by (Carne, 1958).

Christmas beetles are famous for their association with eucalyptus trees. The modified claws in the adults allow them to firmly adhere to leaves (Carne, 1957), and they leave very characteristic “biting” marks in the leaves. Christmas beetles eat eucalypts selectively (Johns *et al.*, 2004; Matsuki *et al.*, 2011) and discriminate based on terpenoids (Edwards *et al.*, 1993) and other volatiles (Matsuki *et al.*, 2011) but can also get confused by introduced species (Steinbauer & Wanjura, 2002). Adults aggregate in great numbers in the same tree to eat and reproduce causing serious defoliation (Johns *et al.*, 2004). Interestingly, beetles can have very specific preferences for certain species, or even varieties of Eucalyptus, but the reasons for

these patterns are not fully understood (Steinbauer & Wanjura, 2002; Johns *et al.*, 2004).

There is uncertainty about the times of the day at which Christmas beetles are active because specimens can be collected during the day feeding or at night in light traps (Steinbauer & Weir, 2007). Overall, it appears they are primarily diurnal forms (Carne, 1974) but they fly after dusk and copulation is frequent at the late afternoon (Carne, 1957). Only *Paraschizognathus* has been reported to be nocturnal. Species in this genus rely on chemical cues to find the females at night since females rarely fly and are very cryptic (Carne, 1974; Steinbauer & Weir, 2007)

- **Colour and vision**

Christmas beetles present some level of intraspecific variation in colour and other optical effects. Species of the genus *Anoplostethus* are the ones with more colour morphs including light blue-green, yellowish green, brownish green, black, bright green, light tan, dark brown, pinkish green, pinkish violet, and mauve. These morphs do not correlate with the locality (Carne, 1958). *Anoplognathus smaragdinus* varies between red, green and blue (**Figure 1-3**). Some other species of *Anoplognathus* that have a green sheen on top of a light brown colour also show intraspecific variation, since the sheen may be present or absent. The ratio of presence/absence of the sheen has not been explored, nor whether this correlates with location.

Meyer-Rochow and Horridge (1975) conducted a very detailed study of the eye of *Anoplognathus*. They found that these beetles have compound superposition eyes with a clear zone below the ommatidia to focus the light on the retina, and tracheoles between the rhabdomeres to enhance photon capture - tracheal tapetum. Both adaptations for a nocturnal or crepuscular lifestyle. However, during the day, the cone cells extend towards the clear zone and pigment is moved to their walls to reduce background noise, and better optomotor responses can be obtained. This could be useful to focus on large objects during the day. Unfortunately, no more studies on Christmas beetles' visual system have been conducted since then.

- **Conservation**

Christmas beetles' populations have been significantly decreasing for the past years (Reid, 2020; Pickrell, 2019). Since Christmas beetles' larvae usually feed on roots of crops, over the past 200 years, agriculture was favourable for them. However, Eucalypt

forests which are the main food resource for the adults have decreased in size since European settlement ([Christmas Beetles - Queensland Museum](#)). Habitat destruction has put a strain on the survival of these beetles. Climate change may be impacting the larvae since they cannot feed if the soil becomes dry and arid and simply not emerge from the pupa (Reid, 2020). Predation by foxes could be another important factor since *Anoplognathus* make up a large portion in fox diet between November to April in Queensland (O'Connor *et al.*, 2019).

Various initiatives exist in Australia to ask for citizens' help in protecting the remaining Christmas beetles populations by avoiding the use of pesticides, planting native grasses and eucalyptus in the garden, and protecting woodlands ([Back Yard Buddies](#), [Xmas beetle app](#) and [name your Xmas beetle](#)). These tools appeal to Christmas beetles' shiny beautiful appearance to increase the interest in their conservation.

1.3 | Thesis and future directions

Why are Christmas beetles pretty? - This would be my answer to anyone asking about the topic of my PhD research - as “simple” as that. My aim was not to discuss any *real* aesthetic value of the beetles' appearances. In fact, the famous quote by M. Hamilton/The Duchess “beauty is in the eye of the beholder” captures the problem of the word “pretty” when considering all the potential natural observers of Christmas beetles. Yet, I use that precise word because of the fascination Christmas beetles have impressed upon me. Thus, more formally, my research topic was: what is the biological relevance of the complex optical effects - visible and non-visible to human eyes-produced by Christmas beetles?

This is not a trivial question for several reasons. Firstly, the complexity of optical effects produced by natural materials has led to heterogeneity in the associated terminology hindering comparisons between studies and disciplines and hampering our efforts to study their biological relevance. Secondly, despite the numerous studies describing the underlying mechanisms of light manipulation, little is known about the subtle or complex modifications needed to produce diversity in closely related organisms, and the potential trade-offs in the optical effects produced by a given structural configuration. Lastly, without considering evolutionary and ecological context, it is almost impossible to understand the biological significance of natural optical effects. My research aims to provide a foundation for understanding the

ecological relevance and evolution of diverse optical effects in Christmas beetles, expecting that some of these concepts and approaches can also be generalised to other organisms.

To address the above-mentioned “not so simple” biological question I divided my project into three different stages: 1) What exactly are the amazing optical effects produced by Christmas beetles (*Chapter 2*); 2) How can beetles produce them? (*Chapter 3*); and 3) Why do beetles need elaborate photonics in their daily lives? (*Chapters 4 and 5*). Perhaps the most important lesson from my PhD is that these three questions are interconnected, iterative and certainly not linear. Unveiling the optical principles and mechanisms of light manipulation can spark stimulating biological questions and large-scale ecological comparisons are powerful tools to inform species selection for photonics studies. Combining these two approaches can accelerate progress in the search for the biological function of striking optical effects.

In *Chapter 2*, I aimed to quantify the diversity of optical effects in Christmas beetles. I proposed a standard set of measurements, parameters and terminology to describe angle-dependent and independent optical effects in visible and NIR spectral bands. This was based on a generalisation of the methods in Gruson *et al.* (2019a). I used Christmas beetles as a case study since their diverse spectral profiles are likely produced by non-ideal nanostructures. The resultant set of measurements and parameters provides a broad picture of the multiple dimensions of colour and NIR reflectance. These dimensions/traits can be studied separately to answer specific biological questions, for example about iridescence and specularity, because these properties can be influenced by different selective pressures. Alternatively, the parameters can be used in combination to provide a detailed description and comparison of optical properties, such as broadband reflectance (e.g. white, grey, black and brown), gold or even iridescence with limited specularity. In *Chapter 2*, I discussed how these parameters can be used to address questions independent from perception, but also how they could be integrated into visual models to address questions where perception is relevant.

In *Chapter 3*, I examined the elytra of three species of green scarab beetles (*Xyloniichus eucalypti*, *Anoplognathus prasinus* and *Paraschizognathus olivaceus*) with high NIR reflectance to identify the underlying mechanisms. I show that these three species have a unique structure that has not been reported in any other species of scarabs. Instead of one single layer of nanostructures, they use a composite material combining

the cuticle which acts like a pigment-based filter, with a structural underlay that produces high broadband (visible and NIR) reflectance. Considering that these three beetles are not sister species, it is possible that the bilayered structures arose due to convergent evolution. This unique bilayer structure is analogous to coloured bilayer coatings for passive cooling, a green alternative to energy-using cooling systems.

The utility of the type of analysis conducted in *Chapter 2* became apparent during the design of *Chapter 3*. The screening of optical properties in *Chapter 2* showed two types of NIR reflectance: broadband diffuse (i.e., low specularity) NIR and iridescent NIR. Angle-dependent effects are rarely studied in the NIR since reflectance in this spectral band is very unlikely to have a visual function (Wang *et al.*, 2022b; Stuart-Fox *et al.*, 2017). I focused on the diffuse NIR reflective species, then looked for disordered structures in the electron microscopy images of these beetles' cuticles, and modelled only those structures to demonstrate how they can reflect diffuse NIR. This approach was much more tractable and effective than obtaining microscopy images for all beetles and modelling all the elements in their complex cuticles. Thus, using the methods in *Chapter 2* to break down complex optical effects into simpler traits facilitated the use of simpler models.

Since Christmas beetles are a relatively small but very diverse group, my original hypothesis was that one mechanism with a few 'tweaks' could explain the variability in optical effects based on the principles for ideal multilayers or diffraction gratings (Starkey & Vukusic, 2013). The reality was more different than I ever expected. My findings in *Chapter 3* (and other microscopy data not included in the thesis) revealed great diversity in the overall architecture of the beetle elytra and the nanostructures in the cuticle. Future studies could further explore the causality between nanostructures and angle dependent effects (*Chapter 2*). For instance, it would be fascinating to explore if NIR iridescence and visible iridescence co-occur in the same organism due to one broad band structure or a stack of two narrowband structures; or to evaluate whether the modifications to produce an iridescent sheen that is completely transparent at certain angles are in the nanostructures, chemical composition, or surface architecture of the elytra. Another exciting opportunity would be to study if bilayered structures (*Chapter 3*) are easier or harder to assemble than chiral structures and if any elements from early stages of development are retained in the adults.

Overall, results from *Chapters 2* and *3* showed that there are multiple ways to achieve high reflectance, a pattern that could be explained by a common selective pressure such as thermoregulation, which may be relevant for Christmas beetles that are active during summer. I combined a physiological (*Chapter 4*) and a macroecological (*Chapter 5*) approach to test this hypothesis. In *Chapter 4*, I designed and used a custom-made device to accurately quantify the small differences in heating between elytra of different species due to solar radiation. In *Chapter 5*, I examined the correlations between the optical properties of Christmas beetles and environmental variables such as temperature, humidity and vegetation cover, accounting for body size and phylogenetic relatedness.

I found that the optical properties of the elytra can predict heating in a biologically significant range (*Chapter 4*); however higher reflectivity (Total and NIR) is not correlated with hotter environments (*Chapter 5*). This suggests that thermoregulation is not a strong driver of the variation in optical properties generally for Christmas beetles. Nonetheless, it may be important for some species with particularly high reflectance or high absorbance, or in species that need to cool down or heat up in specific contexts. Our results from the physiological approach show only the potential effect of the beetle elytra on heat gain. Future studies should be conducted to evaluate the biological relevance of the elytra for thermoregulation, considering the microenvironment and thermoregulatory behaviour of the beetles. The macroecological analyses indicate that humidity may be more relevant than temperatures in determining size and reflectance in the visible spectrum. More studies on the fundamental biology of Christmas beetles are needed to consider how species-specific combinations of ecological challenges can influence their optical effects.

Christmas beetles have at least two very different mechanisms to interact with light. Their chiral arrays (associated with polarisation) are possibly a conserved trait that undergoes modifications between species to create a wide variety of optical effects in the visible spectrum (*Chapter 5*). There is also a trade-off between circular polarisation and NIR reflectance (*Chapter 5*) which could be further investigated. Conversely, the bilayered composite structure in some species seem to be the result of convergent evolution (*Chapter 3*) but the selective pressures driving the evolution of such structures remain to be discovered. Ancestral state reconstructions of the optical traits (described in *Chapter 2*) and mechanisms could reveal whether the evolutionary history of Christmas beetles can explain some observed correlations between their optical properties.

The diversity of visible optical effects in Christmas beetles is astonishing. Angle-dependent and independent effects do not cluster according to genus (*Chapter 2*) and chiral structures enable a wide variety of optical effects in visible wavelengths even in the presence of melanin (*Chapter 5*). Interestingly, in other organisms the presence of melanin deposited below a structure helps to absorb diffusely scattered light to increase iridescence and saturation (Prum & Torres, 2003a; Kinoshita & Yoshioka, 2005), but the biological relevance of underlying melanin in this group of beetles is unclear. The only study on vision in Christmas beetles reported that they have good optomotor responses during daylight but very low visual acuity (Meyer-Rochow & Horridge, 1975), and to the date there are no data on their spectral sensitivity. It seems unlikely that Christmas beetles use visual cues to locate a potential mate. Some optical effects may have a visual function (Miao *et al.*, 2015) but in what species and contexts is still unclear. Another possibility is that some optical effects may be used to avoid predation, though there is conflicting evidence for the role of angle-dependent effects in camouflage (Kjernsmo *et al.*, 2020; Franklin *et al.*, 2021).

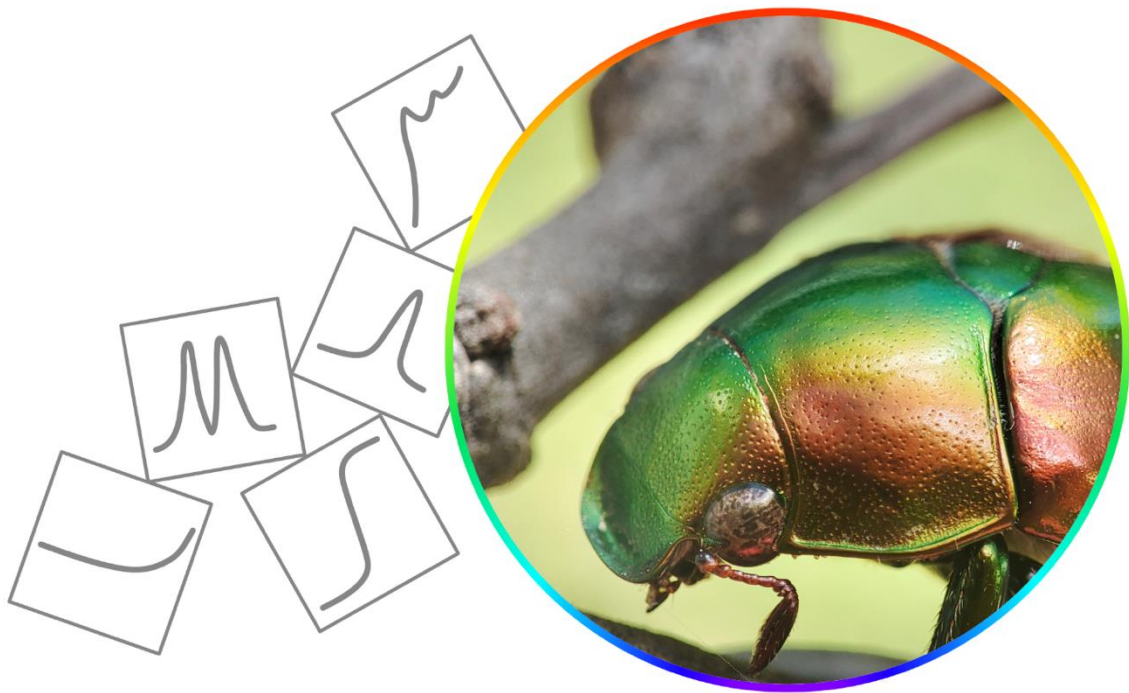
Could the optical effects produced by Christmas beetles be merely a by-product of selection on the structural properties of elytra unrelated to the optical effects they produce? The observed variation in combinations of optical properties in the visible range argues against a single, universal explanation. In addition, total reflectivity in NIR and visible wavelengths are only 58% correlated in this group (*Chapter 4*), indicating that the effects in visible and NIR wavelengths are to some extent decoupled (*Chapter 3*). However, NIR reflectivity and polarisation have high phylogenetic signal (*Chapter 5*). Therefore, it is possible that only some properties are under selection or that only some species have evolved specific combinations of optical properties in response to species-specific challenges. Overall, some traits may be a by-product of selection for other properties, but it is likely that there are different combinations of selective pressures in different species. A next step would be to examine multiple material properties of elytra such as mechanical stability, permeability and hydrophobicity to examine correlations with optical properties such as high structural absorption, chirality and specularity.

Overall, my thesis provided a precise and consistent terminology to refer to the spectral properties of optical effects produced by non-ideal natural materials. In addition, my thesis offered a generalised approach to quantify diversity in optical effects. These two outcomes are useful to study optical traits relevant to specific biological questions. My thesis demonstrated that even in a small group such as

Christmas beetles, there is high diversity in light manipulation mechanisms. Some of these mechanisms entail trade-offs between different optical properties, while some others enable different effects in visible and NIR spectral bands. Finally, my thesis showed that the analysis of multiple phylogenetically related species is useful to examine functional and evolutionary hypotheses.

Chapter 2 - Quantification

A Generalized Approach to Characterize Optical Properties Of Natural Objects



*This chapter is presented as its published version: Laura Ospina-Rozo, Ann Roberts, Devi Stuart-Fox, 2022. [A generalized approach to characterize optical properties of natural objects](#), *Biological Journal of the Linnean Society* 137: 534-555*

2.1 | Abstract

To understand the diversity of ways in which natural materials interact with light, it is important to consider how their reflectance changes with the angle of illumination or viewing and to consider wavelengths beyond the visible. Efforts to characterise these optical properties, however, have been hampered by heterogeneity in measurement techniques, parameters and terminology. Here, we propose a standardised set of measurements, parameters and terminology to describe the optical properties of natural objects based on spectrometry, including angle-dependent effects such as iridescence and specularly. We chose a set of existing measurements and parameters that are generalisable to any wavelength range and spectral shape and we highlight which subsets of measures are relevant to different biological questions. As a case study, we applied these measures to 30 species of Christmas beetles, in which we observed previously unrealised diversity in visible and near-infrared (NIR) reflectance. As expected, reflection of short wavelengths was associated with high spectral purity and angle-dependency. In contrast to simple, artificial structures, iridescence and specularly were not strongly correlated, highlighting the complexity and modularity of natural materials. Species did not cluster according to spectral parameters or genus suggesting high lability of optical properties. The proposed standardisation of measures and parameters will improve our understanding of biological adaptations for manipulating light by facilitating the systematic comparison of complex optical properties such as glossy or metallic appearances and visible or NIR iridescence.

2.2 | Introduction

The way in which living organisms interact with light is fundamental to survival. This interaction gives rise to a wide range of optical effects (a term derived from the field of optics and unrelated to visual perception) such as iridescence and gloss (Shawkey & D'Alba, 2017). Optical properties are often evolutionarily labile and can be simultaneously influenced by different receivers (e.g., conspecifics, predators, prey), context and selection for different biological functions (Kemp *et al.*, 2015; Stuart-Fox & Moussalli, 2009; Cuthill *et al.*, 2017; Endler, 1990). Thus, it is useful to describe the diversity of optical properties independent of specific receivers because animals differ greatly in their visual sensitivity (Kemp *et al.*, 2015; Osorio & Vorobyev, 2008). In addition, optical properties include wavelengths spanning the spectral range of sunlight (~280 - 2600 nm; not only in the 300 - 700 nm UV-VIS region), reflecting light at specific angles, or in various regions of the spectrum at the same time (multi-peaked or sigmoidal spectra) and these complex traits do not necessarily have a visual function. Being able to describe the variability in optical properties without prior knowledge of the underlying mechanism is important for exploring both their visual and non-visual functions, and to understand the evolution of these traits.

Any attempt to characterise optical properties entails simplification, which depends on the measurement approach, nature of the material and biological question. For example, many techniques available to characterise light reflection of natural objects, such as scatterometry and microspectrophotometry (reviewed in Vukusic & Stavenga, 2009), are designed to characterise optical properties of a small region of the surface (<1 mm) and are used in studies describing the underlying mechanism (Vignolini *et al.*, 2013; Wilts *et al.*, 2009; Stavenga *et al.*, 2011; Fu *et al.*, 2017; Kinoshita *et al.*, 2002). By contrast, studies focusing on function and evolution most commonly use spatially unresolved spectrometry to characterise macroscopic optical properties (Johnsen, 2016). This technique involves illuminating and collecting the reflected light at specific combinations of angles (Gruson *et al.*, 2019a). However, most studies measure reflection at only one geometry (e.g. both light source and collection optical fibres within a single probe are oriented at 45° relative to the sample surface), which makes it impossible to distinguish between diffuse and specular reflection (glossary in **Table 2-1**). Diffuse reflection is produced when a surface scatters the incoming light similarly across a wide range of angles; whereas specular reflection is produced when the surface reflects most of the light only at the specular or mirror angle (equal but opposite angle of illumination and reflection; **Table 2-1**). Measurement at one angle

implicitly assumes that the sample is mainly diffuse (i.e., that reflectance is not angle-dependent); however, biological surfaces vary continuously in the relative contribution of diffuse and specular reflection. For example, surfaces with a glossy or metallic appearance have a high proportion of specularly reflected light compared to matte or diffuse surfaces (Stuart-Fox *et al.*, 2020; Franklin & Ospina-Rozo, 2021) (Table 2-1). Many structural colours are also iridescent: changing hue with the viewing or illumination angle (Stuart-Fox *et al.*, 2020; Meadows *et al.*, 2009; Shawkey *et al.*, 2009a; Seago *et al.*, 2009). Angle-dependent effects such as gloss (Table 2-1) and iridescence can be characterised only with angle-resolved spectral measurements (Meadows *et al.*, 2011; Osorio & Ham, 2002; Cuthill *et al.*, 1999). Currently, however, most studies that measure reflection at more than one angle use angles that are not comparable or do not attempt to quantify angle dependency (Gruson *et al.*, 2019a).

Gruson *et al.* (2019a) proposed an effective and straightforward method to characterise the angle-dependent effects of structural colours using spectrometry with an optimized set of geometries and 5 parameters: hue, angle dependency of hue, maximum brightness, tilting and angle dependency of brightness. Their method was derived from the optical theory of multilayers, and was used to quantify diversity in iridescent hummingbird feathers and butterfly wings (Gruson *et al.*, 2019a). Gruson *et al.* suggest that their parameters could be generalisable to characterise angle-dependency produced by other mechanisms. However, the spectral profiles produced by ideal or almost ideal (in birds and butterflies) multilayers are characterised by isolated distinct peaks. Some modifications or additions to the parameters, therefore, are needed to describe angle-dependency in natural materials that produce other spectral shapes including sigmoidal shapes without peaks, broadband spectra and spectra with multiple peaks.

A variety of parameters exist to describe the shape of reflectance spectra. These have been described in various important sources (Gruson *et al.*, 2019a; Montgomerie *et al.*, 2006; Kemp *et al.*, 2015) and can be implemented in the widely used R package *pavo* (Maia *et al.*, 2019). However, inconsistency still exists in both the parameters used to characterise spectral variation, including angle-dependency, as well as the terminology used to describe the parameters themselves (Seago *et al.*, 2009; Galván & Sanz, 2010). This inconsistency arises because multiple parameters can be extracted from each spectral curve and parameters that can be easily calculated for some spectra (e.g. the wavelength at maximal reflectance (Table 2-1) for spectra with single distinct peaks) are not meaningful for others (e.g. sigmoidal spectra). Additionally,

describing optical properties integrates concepts from biology, physics and applied optics but, unfortunately, these disciplines have developed different conventions and terminology (Seago *et al.*, 2009). The current heterogeneity in measurement techniques, parameters and terminology highlights the need for a common set of measurements and parameters to enable accurate and comparable measurements between studies and species.

Here we propose the use of a standardised set of established measurements from spectrometry to capture diffuse and specular reflection as well as angle-dependent properties based on Gruson *et al.*'s approach. As these measures capture macroscopic optical properties, they do not necessarily relate to the underlying mechanism, which is often complex and seldom known for most species (Eliason *et al.*, 2020; White, 2018; Seago *et al.*, 2009; Srinivasarao, 1999). Instead, we demonstrate that the correspondent spectral parameters can be adapted to describe any shape of spectral profile in different regions of the electromagnetic spectrum. We describe which subset of measurements and parameters are relevant to different research questions and material properties. In addition, we propose the use of a consistent set of simple terms to enable comparison between studies, facilitate conceptual unification among disparate fields (biology and optics) and allow a clear distinction with terms used to describe colour perception (psychophysics terms). We emphasise that description of spectral properties cannot tell us about how colour is perceived by an animal, which depends on many factors including photoreceptor sensitivities, colour opponent mechanisms and neural processing (Kemp *et al.*, 2015). However, there are many situations in which it is appropriate to characterise spectral properties independent receiver perception. As a case study, we apply the measurements and parameters to 30 species of beetle in the scarab subfamily Rutelinae, also known as Christmas beetles, which are diverse in their optical effects. These effects may have a role in communication, camouflage and/or thermoregulation in scarabs (Seago *et al.*, 2009; Agez *et al.*, 2017; Thomas *et al.*, 2007), but their biological role for Christmas beetles has not been explored. We measure reflectance at both visible and near-infrared wavelengths, comprehensively characterise spectral properties including angle dependency, and briefly discuss the ecological and evolutionary implications.

Table 2-1. Glossary of terms used to describe spectral reflectance in the literature.

Term	Definition	Alternative terms
SPECULAR	Used to describe surfaces that reflect most of the light at the specular or mirror angle: same magnitude of the incident angle, but opposite direction.	
DIFFUSE	Used to describe surfaces that reflect light over a wide range of angles.	
REFLECTANCE	The proportion of light reflected by the surface at each wavelength interval. It is typically plotted on the vertical axis as a function of wavelength on the horizontal axis to represent a spectral profile and is usually reported as a % relative to a reflectance standard.	Spectral Intensity (Andersson <i>et al.</i> , 2002) Spectral Reflectance (Kemp <i>et al.</i> , 2015)
HEMISPHERICAL REFLECTANCE	Light reflected from a sample over a hemisphere (2π steradians solid angle) is scattered in all directions within an integrating sphere (Figure 2-1). A proportion of the scattered light is collected by a fibre optic connected to a spectrometer.	Overall Reflectance = $2\sqrt{2\pi}B_{max} \gamma_B$ (Gruson <i>et al.</i> , 2019b). Hemispherical/ Plane Albedo (Hapke, 2017) Directional-hemispherical reflectance (Schaepman-Strub <i>et al.</i> , 2004)
HEMISPHERICAL DIRECTIONAL REFLECTANCE	Light is collected from a single angle from a sample illuminated over a hemisphere (wide source). This technique was not used in our study, but we mention it here to contrast with hemispherical reflectance.	Hemispherical Directional (Schaepman-Strub <i>et al.</i> , 2004) Forward Reflectance (Imafuku & Ogihara, 2016)
DIRECTIONAL REFLECTANCE	Light reflected at a specific combination of incident light and collector angles (at a specific geometry).	Bidirectional Reflectance (Schaepman-Strub <i>et al.</i> , 2004; Hapke, 2017)
SPECULAR REFLECTANCE	Directional reflectance at the specular / mirror angle: the collector and incident light are placed at equal angle but opposite direction relative to the normal.	Ordinary reflectance
NON-SPECULAR REFLECTANCE	Directional reflectance at any combination of angles other than the specular angle.	
TOTAL REFLECTANCE	Sum of reflectance over a specified wavelength range. This terms should be used instead of brightness to avoid confusion with the definition of brightness in psychophysics as the perceived	Total Brightness = B_1 in pavo

Term	Definition	Alternative terms
	intensity of light reflected by a surface (Kemp <i>et al.</i> , 2015). Note that the total reflectance can be obtained from any spectra, regardless of the geometry or technique used to capture it.	Spectral intensity (Andersson <i>et al.</i> , 2002)
REFLECTIVITY	The ratio of total reflected to total incident radiation, integrated over the wavelength range of interest (proportion 0 to 1 or %) (Smith <i>et al.</i> , 2016a). Although in the field of optics, reflectivity is used interchangeably with the total reflectance integrated across angles, the distinction between these two terms is particularly relevant in the field of thermal biology (Johnsen, 2012). Consequently, reflectance is wavelength resolved whereas reflectivity as defined here is not wavelength resolved.	
WAVELENGTH AT MAXIMAL REFLECTANCE	The wavelength at which reflectance is maximal. This term, or spectral location (Table 2-2), should be used instead of hue to avoid confusion with the definition in psychophysics as the perceptual dimension describing the category of colour, which is not only determined by the spectral shape, but also by visual and processing features of the observer.	Hue = H1 in <i>pavo</i>
GLOSS	Specularity that gives rise to multiple visual effects depending on the spread of reflected light around the specular angle (Franklin & Ospina-Rozo, 2021).	

2.3 | Methods

2.3.1 | Generalisable measurements, parameters and terminology

The two subsections below describe the measurements needed to capture angle-independent and angle-dependent reflectance as well as recommended terminology.

- **Hemispherical reflectance**

We use this term to refer to spectral measurements where reflected light is integrated across angles in a complete hemisphere. This includes both diffuse and specular reflectance (Table 2-1 and Figure 2-1), therefore it does not provide any information regarding angle dependent effects. It is often called total or overall reflectance (Gruson *et al.*, 2019b), but we recommend the term hemispherical reflectance to differentiate this parameter from the area under a given spectral curve, which is also generally termed total reflectance (see reflectance and total reflectance in Table 2-1).

- **Directional reflectance**

We use this term to refer to angle-specific spectral measurements used to describe angle-dependent properties (Table 2-1). We distinguish two types of angle-dependent change in the spectral profile of a surface: 1) iridescence: an angle-dependent change in wavelength (here we use spectral location to describe the change in wavelength; Table 2-2) and 2) specularity: an angle-dependent change in mean reflectance (Table 2-2). Specularity ranges from a perfect mirror where all light is reflected at the specular angle and none at other angles, to a perfectly diffuse surface (zero specularity) where light is reflected equally at every angle.

To analyse angle-dependent properties, it is necessary to use a spectrometer coupled to a goniometer (schematic in Figure A-1) to consider multiple measurement geometries. Measurement geometry describes the specific configuration of the angle of incident light and angle of the collector, relative to the normal (perpendicular to the sample surface). We followed the procedure proposed by Gruson *et al.* to extract the maximum information from a minimum set of measurement geometries (see detailed explanation in Gruson *et al.*, 2019a). They used two variables to describe each geometry: 1) the span, as the angle between the direction of illumination and the direction of the collector; and 2) the bisector, as the line that divides the span into

two congruent angles (Figure 2-1). Here we use these two terms to identify the two sets of measurements (schematics in Figure 2-1) to capture directional reflectance:

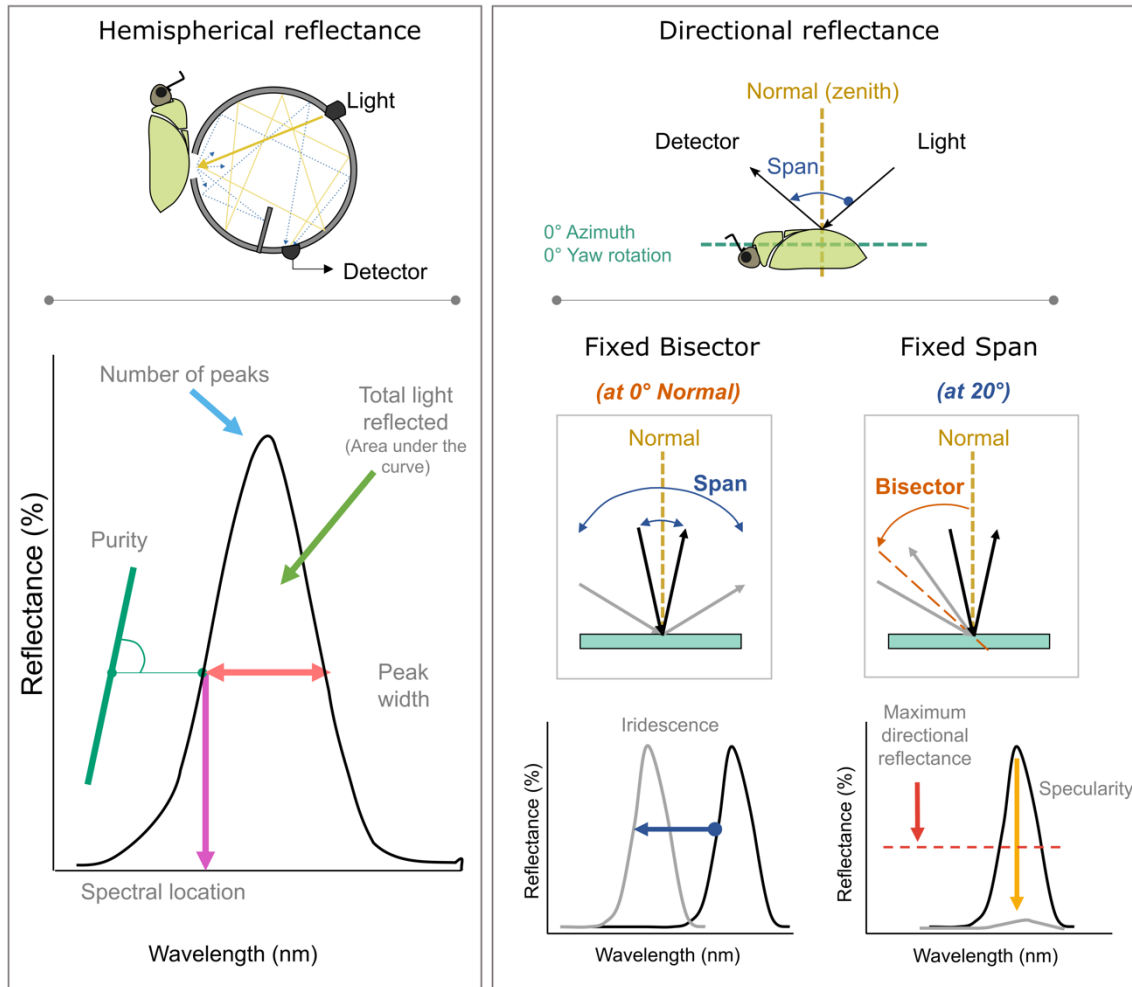


Figure 2-1. Methods and parameters to characterize reflectance

Top panels, measurement techniques. For hemispherical reflectance the reflected light is integrated across angles. For directional reflectance the span is the angle between the light and collector fibres; the bisector is the line that divides the span in half, and it may be aligned or away from the normal (zenith). The azimuth and yaw describe the alignment between the longitudinal axis of the sample and the plane of the spectrometer (see more details in Figure A-1). Left column: parameters from hemispherical reflectance. Number of peaks and peak width are also represented here since they are angle independent. Right column: the fixed bisector was used to calculate iridescence and the fixed span to calculate specularity and maximum directional reflectance (mean reflectance at the specular angle).

- *Fixed bisector set*: This configuration captures the light reflected at the specular or mirror angle (see specular reflectance in Table 2-1) by keeping the bisector constant at 0° (normal to the sample) and adjusting the span to 20° , 30° , 40° , 50° and 60° . These geometries keep the reflectance constant at the maximum to specifically test for iridescence whereby the wavelength or spectral location shifts with the span. This assumes that the surface of the sample is parallel to the light reflecting structure (e.g. multilayers), which may not always be the case. If the structure is heavily tilted relative to the surface of the sample, then the sample tilt would need to be adjusted until reflectance is at the maximum. However, this can be time consuming and, in many cases, it is sufficient to place the sample horizontally.
- *Fixed span set*: This configuration enables comparison between specular and non- specular reflectance (Table 2-1) since it captures the light reflected at the specular angle and at a representative range of angles away from it. This is achieved by keeping the span constant at 20° while altering the elevation (angle with respect to the normal / zenith) of the bisector from -30° , -20° , -10° , 0° , 10° , 20° , and 30° around the normal. These geometries keep the spectral location fixed while isolating the effect of specularity.

This combination of measurement geometries, originally proposed by (Gruson *et al.*, 2019a) is sufficient to describe iridescence and specularity, although it does not permit discrimination between different types of gloss (Franklin & Ospina-Rozo, 2021), for which measurements at angles in very close proximity to the mirror angle are needed. Two additional parameters should be reported: the azimuth and yaw angle (**Figure A-1**). In our set up, the anteroposterior longitudinal axis of the sample and the plane of the two fibres (light and collector) are aligned (azimuth = 0 and yaw = 0, Figure 2-1). Rotating the sample so that its longitudinal axis is at a different angle from the plane of the spectrometer and light source would change the yaw angle. Keeping the azimuth at 0° (zenith) ensures the equipment remains properly aligned in this plane (see details in **Figure A-1**). In both the fixed bisector and fixed span sets, the elevation of the light and collector probes changes. In the fixed bisector set, both probes change symmetrically, while in the fixed span set their position is better described by the elevation of the bisector itself (Figure 2-1).

■ Parameters

We implemented a set of parameters based on simple statistical descriptions of spectral profiles (Figure 2-1). In order to capture the chromatic characteristics of the sample, we used the number of peaks; spectral location (defined as the wavelength at half maximum reflectance); and iridescence. We used two measurements to describe saturation: peak width and spectral purity (a measure of curve steepness) because they vary differently for peaked and sigmoidal spectra (details in Table 2-2 and **Table A-6**). We used three different measurements to capture the achromatic properties of reflectance: the total reflectance for hemispherical reflectance, the maximum directional reflectance and specularity (angle-dependent change in mean reflectance). All these parameters were also applied to NIR reflectance. We examined the relationship between NIR and visible wavelengths by calculating the ratio of NIR/VIS reflectance from the hemispherical reflectance measurements. The description of and rationale for each parameter, as well as equivalent terms, are detailed in Table 2-2. Advantages and limitations of the parameters we have chosen compared to others are discussed in more detail in the Discussion section. The parameters used here can be obtained directly from the widely used R package *pavo* (Maia *et al.*, 2019) or calculated with simple functions (details in Table 2-2 and the R functions [here](#)).

The terminology we advocate for the parameters avoids using psychophysics terms related to perception, such as hue, chroma and brightness (Kemp *et al.*, 2015; Table 2-1). We recognise that the terms saturation and spectral purity overlap with psychophysics concepts but retain them as statistical descriptors here because they are also widely used to describe spectral characteristics independently of perception.

Table 2-2. Parameters to describe reflectance properties used in this study

Parameter	Implementation	Alternative terms	Justification
TOTAL HEMISPHERICAL REFLECTANCE Integrated hemispherical reflectance (% reflectance × nm)	Area under the reflectance curve (i.e., total reflectance) calculated for the hemispherical reflectance spectrum. This measurement is wavelength resolved and integrated across angles since it is calculated from the spectra measured with an integrating sphere.	Total Brightness (Gruson <i>et al.</i> , 2019a) Spectral intensity (Andersson <i>et al.</i> , 2002) B1 in <i>pavo</i>	Total reflectance integrated over directional reflectance measurements tends to have low repeatability. When calculating it from hemispherical reflectance, results are reliable (Table A-2).
SPECTRAL LOCATION The wavelength at which reflectance is half of the maximum (nm)	Wavelength at half maximum reflectance. For a spectrum with peaks, the spectral location was always taken from the shorter wavelength side of the peak. For a spectrum with more than one peak, we calculated this parameter for the peak with shorter wavelength. For a sigmoidal spectrum, this corresponds to the cut-off wavelength normally associated with redness. Calculated from both hemispherical and directional reflectance spectra.	Hue or redness (Andersson <i>et al.</i> , 2002) $Loc = H1 - HWHM$ in <i>pavo</i> instead of H3. Where H1 is the wavelength at maximal location (nm) and HWHM is the half width of the peak at half maximum reflectance (nm)	While the wavelength at maximal reflectance is useful to describe peaked spectra, it is not able to differentiate between non-saturated or sigmoidal spectra (red, brown, pink, white, yellow), for which it tends to be always 700nm. Spectral location can be used to characterize spectra with and without peaks.
SPECTRAL PURITY The standardised difference between integrated reflectance on either side of the spectral location (dimensionless quantity. Range: (0:1))	$C_{R50} = \frac{(R_{\lambda_{min}-\lambda(R50)} - R_{\lambda(R50)-\lambda_{max}})}{R_{\lambda_{min}-\lambda_{max}}}$ Where R represents the total reflectance (area under the curve) across a given range of wavelengths and λ_{R50} = spectral location of a given sample. Values close to 0 indicate lower spectral purity. In this study we used λ_{min} = 400 nm and λ_{max} = 700 nm for visible light, and λ_{min} = 700nm and	Chroma (Andersson <i>et al.</i> , 2002) Similar parameters in <i>pavo</i> : S2 (spectral saturation) and S8 (chroma) (Maia <i>et al.</i> , 2019)	This parameter was used in addition to the peak width, even though they are both considered an indication of saturation. We do not consider them interchangeable, since they are only weakly correlated.

Parameter	Implementation	Alternative terms	Justification
	$\lambda_{\text{max}} = 1400 \text{ nm}$ for NIR. This value was calculated from the hemispherical reflectance spectrum.		
NUMBER OF PEAKS (dimensionless quantity. Range: [0:2])	We manually counted the peaks evident in most of the measurements of hemispherical and directional reflectance for each species. We did not consider a sigmoidal shape a peak; however, when a broad peak in the visible extended to the NIR, it was still considered a peak.		We evaluated the presence of peaks based on both types of measurements (hemispherical and directional reflectance) since the integration across angles in hemispherical measurements can desaturate the spectra and mask peaks.
PEAK WIDTH Full width of the peak at half maximum reflectance (nm)	Calculated for directional reflectance spectra at 40° span. For spectra with multiple peaks, we averaged the width of the peaks. We assigned a value of 0 if the number of peaks was 0.	Saturation (Gruson <i>et al.</i>, 2019a) Full width at half maximum (FWHM) (nm) in <i>pavo</i>.	This parameter is not angle-dependent (Gruson <i>et al.</i> , 2019a), so we measured it for only one geometry of directional reflectance. However, it can differ when calculated from hemispherical reflectance spectra because, for iridescent samples, the integration across angles gives an average reflectance that is less saturated than the directional reflectance.
IRIDESCENCE Angle dependent shift in wavelength. Here we measured it as the angle dependency of spectral location: shift in the spectral location towards shorter wavelengths (blue-shift) with an increase in the span angle (incident and observation angle) (nm/ 1° of span)	We fitted a linear model to describe the change in spectral location explained by the change in span. We calculated iridescence as the negative of the slope of this linear model, so that iridescence corresponds to the extent of blue-shift. Higher values indicate more iridescence, values close to 0 describe samples without iridescence and negative values describe samples with red shifting. The term blue shift is a widely used abbreviation in the field of optics and photonics, which describes a shift of any spectra towards shorter wavelengths and it is not restricted to spectra centred on wavelengths around 450 and 495 nm. Similarly, the term red shift, refers to any shift in the spectra towards longer wavelengths.	Angle dependency of hue (Gruson <i>et al.</i>, 2019a; Gruson <i>et al.</i>, 2019b) Spectral richness (Deparis <i>et al.</i>, 2008)	Iridescence follows a cosine model in multilayers (Gruson <i>et al.</i>, 2019a). However, we used a linear model as a simplified statistical descriptor. The fit of a sinusoidal and linear model should be comparable for the narrow range of angles used here, and in samples with relatively simple structures (see Discussion and Figure A-6). For other situations, the cosine model is more appropriate. We calculated iridescence based on spectral location to be able to analyse sigmoidal spectra and to improve sensitivity for spectra with broad band peaks (see Spectral Location; also Figure 2-3).

Parameter	Implementation	Alternative terms	Justification
SPECULARITY Angle dependency of mean reflectance: a higher value means that most of the light is reflected at the specular angle ($1/^\circ$)	Mean reflectance is calculated as the sum of reflectance over the wavelength range of interest divided by the number of wavelength intervals. We fitted a Gaussian model to describe the mean reflectance value explained by the position of the bisector divided by 2. The model follows the equation: $D(\delta) = D_{max} \times \exp\left[-\frac{(\delta/2 - t)^2}{2\gamma^2}\right]$ Where $D(\%)$ is the mean reflectance at any given position of the bisector. $\delta(^\circ)$ is the position of the bisector. i.e. the difference between the angle of illumination and the angle of the collector in (Gruson <i>et al.</i>, 2019a). $D_{max}(\%)$ is the maximum directional reflectance produced by the surface (at 20° span). The parameter $t(^\circ)$ is a correction to account for the potential imperfect alignment of the structures with the surface of the sample. And $\gamma(^\circ)$ indicates the spread of the reflection of the light away from the mirror angle. This term also represents the deviation or width of the gaussian (also called “RSM width”) named γ_B in (Gruson <i>et al.</i>, 2019a). We calculated specularity as $S = 1/\gamma$. High values of specularity correspond to small spread of the reflection of the light away from the mirror angle, so a greater drop in reflectance when the bisector moves away from the mirror angle.	Angle dependency of brightness (Gruson <i>et al.</i>, 2019b) Directionality $S = 1/\gamma_B$ (Gruson <i>et al.</i>, 2019b) (Gruson <i>et al.</i>, 2019a) Gaussian model between the mean reflectance values, B2 in <i>pavo</i> for each bisector angle.	To estimate specularity we used the formula for angle dependency of brightness described in (Gruson <i>et al.</i>, 2019a). The parameter γ_B was originally described as proportional to unspecific irregularities in multilayers. However, since it directly measures the spread around the specular /mirror angle, it is expected to be an indicator of irregularities in any structure/natural object. For all cases this parameter should be proportional to diffuse reflection and therefore inversely proportional to specularity.
MAXIMUM DIRECTIONAL REFLECTANCE Maximum directional reflectance at any	The maximum value of mean reflectance that can be obtained from a surface after considering different geometries. It is likely to occur at the mirror angle. Mean reflectance is calculated as the sum of reflectance over the wavelength range of	B_{max} (Gruson <i>et al.</i>, 2019a)	This parameter should not be confused with the maximum relative reflectance B3 obtained in <i>pavo</i>, since the latter refers to the maximum value of the y axis in any spectral profile.

Parameter	Implementation	Alternative terms	Justification
geometry, usually at the mirror angle (%)	interest divided by the number of wavelength intervals. The constant D_{max} in the Gaussian model used to calculate specularity refers to the height of the curve's peak. It is the estimate of the maximum amount of light (averaged across wavelengths, B2 in <i>pavo</i>) reflected as directional reflectance by a surface. We created a function to obtain a at 20° span (since our fixed span set was taken at this angle for consistency with Gruson et al.'s approach)		Although this value is correlated with specularity, we included it as a separate parameter since it can also be affected by absorbance and contrast gloss (Franklin & Ospina-Rozo, 2021) producing very different optical effects.
NIR/VIS RATIO Ratio of integrated reflectance in Near infrared and visible wavelengths (Proportion)	$Ratio = \frac{R_{700nm-1400nm}}{R_{400nm-700nm}}$ Where R represents the total reflectance (area under the curve) across the indicated range of wavelengths. Calculated from hemispherical reflectance.		Since reflectance in visible and NIR wavelengths are usually correlated, this measurement is useful to detect variation in relative reflectance in the two wavelength ranges.

2.3.2 | Christmas beetles as a case study

- Species

We studied 35 scarab beetle specimens belonging to 30 species from the subfamily Rutelinae (Scarabeidae) also known as Christmas beetles (Table A-1). The specimens included green, red, and purple morphs of *Anoplognathus smaragdinus*; bronze and green sheen morphs of *A. porosus* and black and green subspecies of *Repsimus manicatus*. All specimens were sourced from the Australian National Insect Collection ANIC / CSIRO (Table A-1).

- Spectral measurements

In order to measure hemispherical reflectance, we used an integrating sphere with an inbuilt tungsten-halogen light source (400-2100 nm; ISP-REF; Ocean Optics Inc., Dunedin, FL, USA.) and 4 mm diameter sampling port. The integrating sphere was connected to two spectrophotometers, the USB 2000+ (400-1000 nm) and NIRQuest (1000-2100 nm) via a bifurcated optic fibre. Measurements for visible and NIR were recorded simultaneously and stitched together using the software OceanView 1.6.7 before calibration. We measured one elytron on each specimen without detaching it from the body. We repeated the measurement of each sample four times on different days and averaged the results.

For directional reflectance measurements, we used the same two spectrometers as for the hemispherical reflectance measurements and two light sources: a PX-2 pulsed Xenon light for the UV-visible range and HL-2000 tungsten halogen light for the visible-NIR range (both from Ocean Optics Inc., Dunedin, FL, USA). The spectrometers and light sources were coupled to a goniometer, which allows precise control of the angle of incident light and collector (Figure A-1). The goniometer was part of a custom designed set-up that allowed simultaneous measurement of visible and NIR reflectance over the same 1mm diameter spot size. The spectra from the UV-Vis and NIR spectrometers were stitched together using the software OceanView 1.6.7. We measured one spot in a 3mm x 3mm piece of elytron fixed on a microscope slide. We repeated the measurement of each sample three times on different days to reduce the noise from random measurement error and averaged the results. Modifying the angles of the collector and light source inevitably alters the size of the spot (the spread of the beam changes as well as the area illuminated) and the optimal location of the

sample. In order to account for this, every time the geometry was modified, we checked and adjusted the position of the standard and sample and recalibrated.

Choice of standards: in both cases, for hemispherical and directional reflectance we calibrated against a Lambertian 99% reflectance spectralon standard (Labsphere, North Sutton, NH, USA). Given that some of the beetles in our sample were highly specular, we frequently obtained measurements above 100% for directional reflectance measurements, particularly at the specular angle. This is because the sample reflects more light at the specular angle than the diffuse standard, which scatters the directional illumination equally across all angles. Hence, reflectance values above 100% are not problematic if all samples are compared to the same standard. For highly specular samples, a specular standard may be required to ensure a sufficient signal to noise ratio for accurate measurements in the fixed bisector set (in which all measurement geometries are specular); however, for most biological samples and non-specular measurement geometries (i.e. fixed span set), a diffuse standard is appropriate. If different standards are used for different measurements, post hoc mathematical conversions between standards can be applied.

- **Wavelength range and generalisation**

For both hemispherical and directional reflectance measurements, we excluded UV wavelengths because of the limitations of the inbuilt light source of the integrating sphere and because Christmas beetles have very low reflectance below 400 nm. However, the methods we propose here can easily be extended to UV wavelengths if required. In addition, some Christmas beetle species only reflect left-handed circularly polarized light. The equipment we used can characterise the spectral properties of this light, but not its polarization. Polarization was beyond the scope of this study, but these methods could be extended to incorporate polarization effects by including linear or circular polarization filters placed in front of the collector and/or light source probe. We measured the optical effects at only 0° azimuth, but for experiments with samples with certain directionality (for example hairs or scales) it may be important to take the same measurements in more than one azimuth angle.

- **Repeatability**

We evaluated repeatability as the agreement of successive repetitions of the same measurement (details in **Table A-2**) for the following parameters: spectral location and total hemispherical reflectance (VIS and NIR) and spectral location and mean

reflectance in directional reflectance measurements. Repeatability was high for all the parameters, ranging between 0.82 and 0.99. We also calculated the standard deviation (**Table A-2**) to estimate the median and range of the variability for each parameter. Estimates for the standard deviation of the spectral location are between 1 to 4 nm in hemispherical reflectance (**Table A-3**) and hemispherical and 1 to 8 nm in directional reflectance (**Table A-4**). The total reflectance varied with a median of 1.7 to 2.8 % (sd standardized by the mean, i.e. CV) in hemispherical reflectance (**Table A-3**) and the mean reflectance varied between 1.6 to 62 % (CV) in directional reflectance (**Table A-5**). In the latter configuration, variation can be very high for some species. These results are not due to lack of reliability of the setup, but due to the properties of the samples. Specular samples tend to have more variation because the positioning of the sample has a considerable effect on the light collected by the collector. Our approach accounts for this variation by using a fitted value as the maximum directional reflectance: the amplitude of the Gaussian equation that describes the change in mean reflectance according to the measurement geometry (details and equation in Table 2-2).

- **Application of the method and statistical analysis**

In order to examine the pairwise correlations in parameters for this group of scarabs, we used Pearson's correlations between parameters for visible and NIR wavelengths separately. We visualized the differences in optical appearance between the 30 species of Christmas beetles using a principal component analysis (PCA). We conducted two independent PCAs, one for visible and one for NIR wavelengths, since we hypothesized light manipulation in these two wavelength ranges may be subject to different selective pressures (Smith *et al.*, 2016a), and thus vary in different ways between species. The PCA enabled us to determine if there was clustering in relation to optical properties or taxonomy (genus).

2.4 | Results and discussion

We propose a combination of measurements and parameters to enable reliable and repeatable estimates of both diffuse and specular reflection for natural materials that can be comparable across study questions and systems. The selected parameters can be applied to any spectral profile, including broadband, sigmoidal and multi-peaked spectra in different regions of the electromagnetic spectrum, which is useful for addressing non-visual functions, underlying mechanisms and quantifying diversity

(Figure A-2, Figure A-3, Figure A-4 and Figure A-5). The approach advocated here combines and extends established techniques; specifically, measuring hemispherical reflectance (Johnsen, 2016), measuring directional reflectance with a goniometer (Meadows *et al.*, 2011) and implementing an optimized set of geometries to characterize angle-dependency proposed by Gruson *et al.* (Gruson *et al.*, 2019a). The parameters are commonly used to describe spectral profiles (Montgomerie *et al.*, 2006) and can be calculated using the widely used R package *pavo* (Maia *et al.*, 2019). Although these parameters are statistical descriptors, they are consistent with optical principles and definitions from physics (Johnsen, 2012). We provide a standard nomenclature, consistent with recent attempts to improve uniformity of terminology in the biological literature (Kemp *et al.*, 2015; Stuart-Fox *et al.*, 2020) and with terminology used in physics (Vukusic & Stavenga, 2009; Schaepman-Strub *et al.*, 2004; Hapke, 2017). The nomenclature is largely concordant with the names of the parameters in *pavo* (Maia *et al.*, 2019); however, we propose some changes to avoid overlapping with psychophysics (Table 2-2).

- **Parameters - advantages and limitations**

We applied the methods to describe angle-dependency proposed by Gruson *et al.* (Gruson *et al.*, 2019a), with some adaptations so that they can be used to describe the full range of spectral profiles. First, we used spectral location rather than the wavelength of maximal reflectance and calculated iridescence as the shift in spectral location as a function of angle because these two measures can be applied to sigmoidal and broadband spectra. However, when using spectral location, it is important to verify that the saturation is not changing with the change in angle, otherwise, desaturation could be mistakenly interpreted as iridescence. Saturation is constant in the angles of the fixed bisector set for multilayers (Gruson *et al.*, 2019a), but in other combinations of angles, or organisms with complex, composite mechanisms, saturation should be measured.

Second, we used a linear rather than sinusoidal regression model to estimate iridescence (i.e., the shift in spectral location as a function of angle). Iridescence produced by multilayers results in a spectral shift towards shorter wavelengths with increasing angle following a cosine function (Gruson *et al.*, 2019a), although other mechanisms may produce a different pattern. For the relatively narrow range of angles considered here, the shift is approximately linear (Figure A-6 and Table A-6). The advantage of using a linear model for non-ideal structures is that the slope is easily interpreted as the shift in nm per degree. The slope also indicates the direction of the

spectral shift: negative = blue shift, positive= red shift, 0 = no iridescence; whereas a sinusoidal model is not applicable to the latter two cases (**Figure A-6** and **Table A-6**). However, a linear regression is not appropriate to predict spectral location beyond the interval of span angles recommended here: between 20° and 60°. Doing so would overestimate iridescence. We highlight that a cosine function (Gruson *et al.*, 2019a) is more appropriate to accurately characterise iridescence in multilayers and a linear fit is a simplification that should be used carefully.

Third, we included two different measures of saturation, spectral purity and peak width. Strongly peaked spectra are highly saturated based on both measures (high spectral purity and narrow peak width), whereas high saturation for sigmoidal spectra or very broad band peaks can be characterised only based on spectral purity. Additionally, some spectra include both sigmoidal and peaked components. One may therefore choose to use one or both of these measures depending on the spectral properties of the study system.

Lastly, we note that characterisation of multi-peaked spectra is an enduring problem (Noh *et al.*, 2010; McGraw, 2004). The number of peaks was calculated manually in our study. Unfortunately, obtaining this parameter from spectra cannot be easily automated, and it is inevitably subjective since peaks (maxima) are not always clearly defined. However, we applied consistent criteria and considered a peak only if it was present in all 13 spectra for each beetle. Automation of peak identification using a machine learning or improved optimisation algorithm warrants further investigation. This parameter can be excluded if the samples studied do not produce multi-peaked spectra.

▪ Measurements and parameters for different research questions

For any given study, the appropriate choice of measurements and parameters will depend on the object properties and research question. Hemispherical reflectance is relevant for studies of thermoregulation because it represents the proportion of light (visible or near infrared) not absorbed or transmitted by the sample. This measurement can be used in studies of visual functions, only if the surface is diffuse, or if the goal is to contrast the main wavelengths reflected against the background. Hemispherical reflectance can be used alone if angle dependent effects do not need to be considered in the study. Conversely, directional reflectance is useful in studies that need to consider angle dependent visual effects such as iridescence, gloss and intensity flashes, which have been associated with both communication and camouflage (Stuart-

Fox *et al.*, 2020; Franklin & Ospina-Rozo, 2021). Directional reflectance measures comprise the fixed bisector and fixed span sets. The fixed bisector set isolates the effects of iridescence and is ideal for studies on the biological relevance of iridescence. The resulting spectral measurements can be used in visual models for studies on visual functions of iridescence (Hogan & Stoddard, 2018; Girard & Endler, 2014; Kjærsmo *et al.*, 2020). The fixed span set isolates the effect of specularity and can be used to study the biological relevance of intensity flashes, high specularity or gloss (Franklin *et al.*, 2021; White *et al.*, 2015). Lastly, NIR reflectance is relevant for thermoregulation rather than visual functions; therefore, the relevant parameters are total reflectance and NIR/VIS ratio obtained from hemispherical reflectance (Bakken *et al.*, 1978; Medina *et al.*, 2018). Directional reflectance in NIR is primarily relevant for studies focusing on mechanisms to reflect NIR light; for example, iridescence is a hallmark of multilayers (Starkey & Vukusic, 2013), whereas broadband, non-iridescent, non-specular NIR reflectance may be produced by disordered arrays (Johansen *et al.*, 2017).

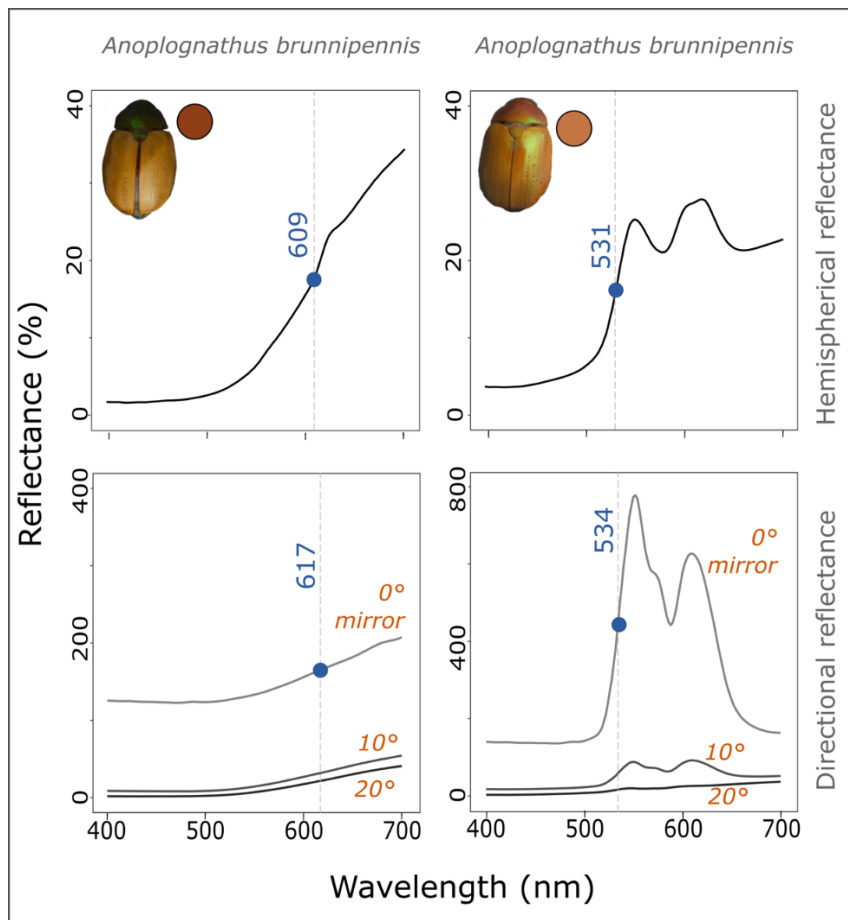


Figure 2-2. Examples of the diversity detected by the combination of hemispherical and directional reflectance

The hemispherical reflectance profiles are similar: reflectance increasing towards longer wavelengths. The calculated RGB colour is brown based on human vision models (circles). The

directional reflectance profiles are different: *A. brunnipennis* has a redder spectral location and *A. pindarus* has two clearly defined peaks with very high reflectance only at the mirror angle (bisector angle = 0, in orange). Both beetles have spectral profiles above 100% at the mirror angle. In *A. brunnipennis* it is produced by the gloss of the outer layers of wax or the epicuticle, while in *A. pindarus* it is produced by the specularity of the structural colour. The spectral location (blue) is more effective for capturing the difference in specular reflectance than the wavelength of maximum reflectance.

- **Case study: diversity of light manipulation in Christmas beetles**

We have shown that the combination of techniques we used was appropriate to describe and detect different combinations of pigment-based and structural coloration in Christmas beetles, organisms that are more likely to have optical effects arising from modular complex structures rather than from ideal multilayers (**Figure A-2, Figure A-3, Figure A-4 and Figure A-5**). For example, the hemispheric reflections of the two beetles in Figure 2-2 are very similar, showing a pattern associated with a pigment-based brown colour. However, they are very different in their directional reflectance profile which shows two irregular peaks in *A. pindarus* likely to be produced by an overlying multilayer structure. This example also highlights the utility of using spectral location rather than the wavelength of maximum reflectance. The latter can easily differentiate spectra with well-defined peaks but fails to show differences for sigmoidal spectra. Spectral location is more effective in capturing these differences (Figure 2-2).

We found that angle-dependent properties varied substantially among species for both visible (Figure 2-3) and NIR (Figure 2-4) wavelengths, altering the overall optical effect in different ways. For example, both *A. aureus* and *C. frenchi* have strong iridescence of around 20nm blue-shift with the change from 20° to 60° span, comparable to the iridescence of the hummingbird *Helimaster furcifer* (~25 nm blue shift for the same change Gruson *et al.*, 2019a). However, to the human eye, *C. frenchi* clearly changes from red to deep green with an increase in the viewing angle, while *A. aureus* does not appear to change. This effect occurs because the iridescence in *C. frenchi* is intensified by the presence of two peaks (both blue shifting), while the iridescence of *A. aureus* may be masked to the human eye by its broadband spectrum (Mitov, 2017). The gold-like metallic appearance of *A. aureus* is explained by the high degree of specularity (Figure 2-3).

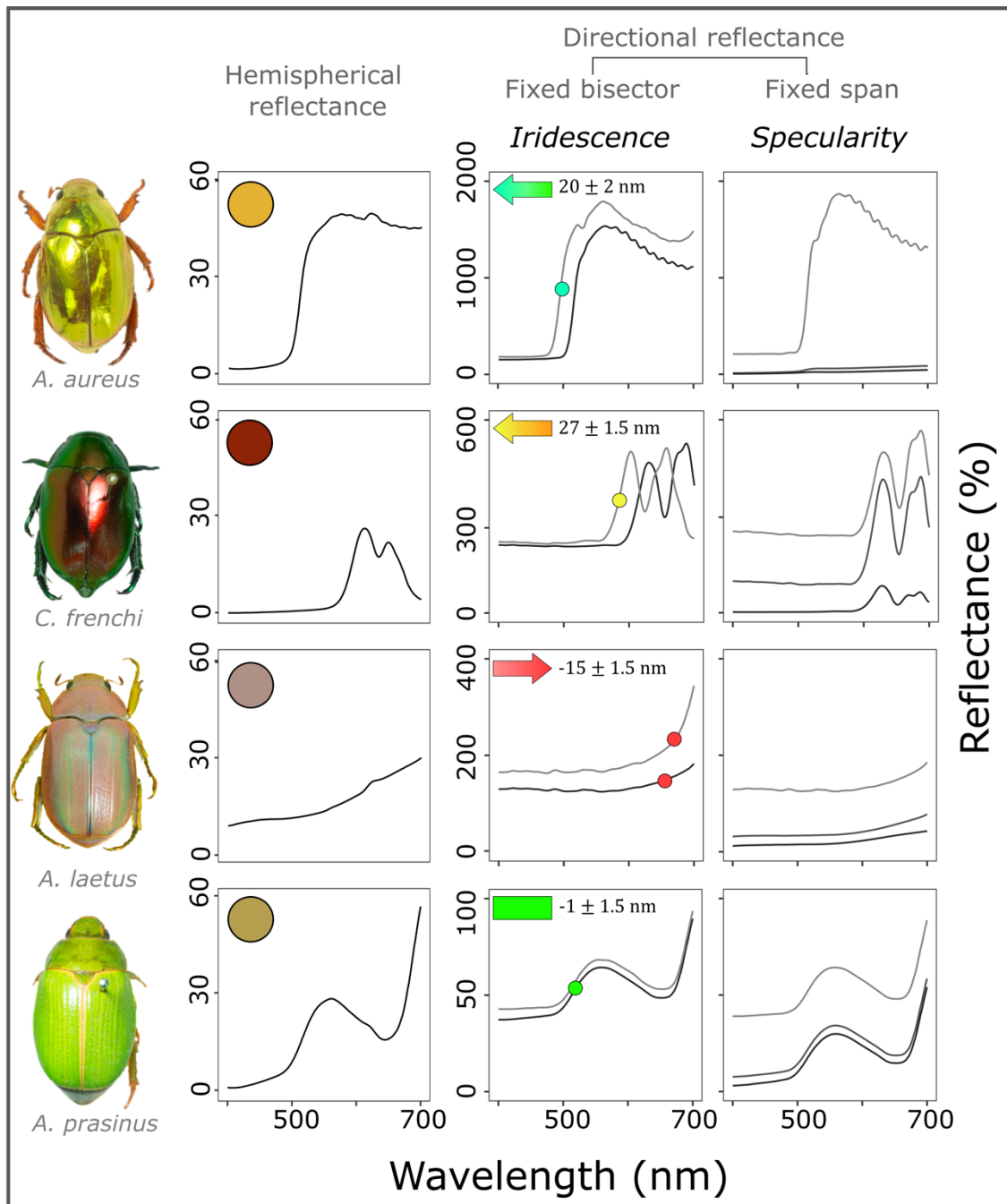


Figure 2-3. Diversity of angle dependent properties

Four representative beetles are shown. The circles within the hemispherical reflectance panels show the calculated RGB colours based on human vision. The examples show different magnitudes of iridescence, reverse iridescence (red shift in *A. laetus*) and no iridescence (*A. prasinus*). Arrows show the direction and change in nm in spectral location (circle in the profile) in response to a change in geometry from 20° (black line) to 60° (gray line) span. The examples also show different values of specularity (note y axis). High specularity is a considerable reduction in reflectance for bisector angles other than 0° (bisector = 0° light gray, 10° dark gray, and 20° black).

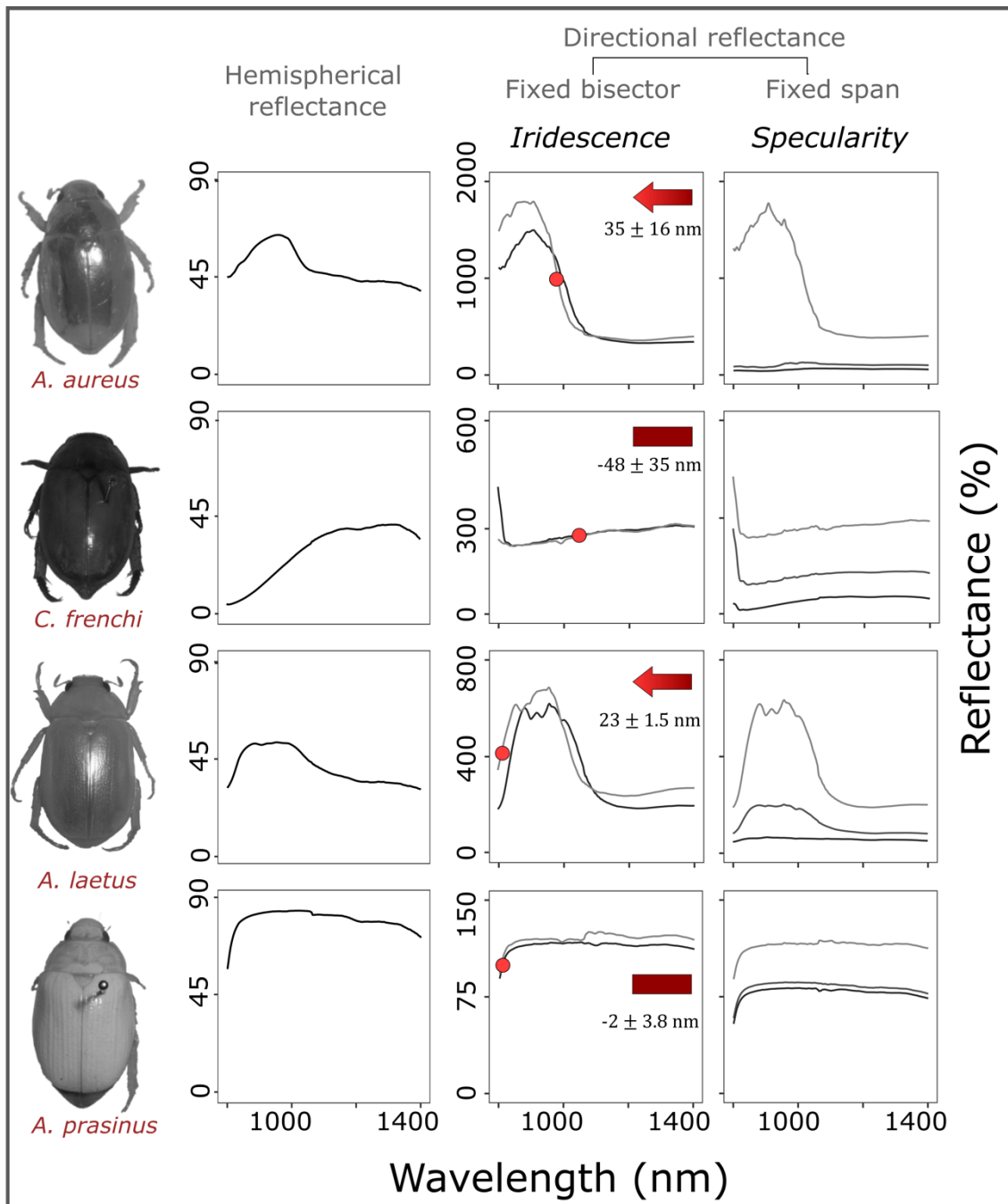


Figure 2-4. Diversity of NIR reflectance

NIR spectra of the same species as in Figure 2-3 with calibrated NIR photos on the left. The examples show different magnitudes of NIR iridescence. Arrows show the direction and change in nm in spectral location (circle in the profile) in response to a change in geometry from 20° (black line) to 60° (gray line) span. The examples also show different values of specularity - a considerable reduction in reflectance for bisector angles other than 0° (bisector = 0° light gray, 10° dark gray, and 20° black). The high iridescence and specularity observed in *A. aureus* and *A. laetus* explain the metallic appearance of these beetles in NIR calibrated photos while the broad band profile of *A. prasinus* explains its white appearance in NIR.

Another interesting example is *A. laetus*, which appears pearlescent to the human eye: pink at the periphery and pale blue in the centre (Figure 2-3). We discovered that this is the result of “inverse iridescence”, since *A. laetus*’ spectral location appears red shifted with increasing span. Inverse iridescence can be produced by a modular

combination of structures (Vukusic *et al.*, 2002), but this is not the case for *A. laetus*. Instead, this beetle has a clear peak in reflectance in the NIR with a high degree of iridescence (Figure 2-4) which gradually enters the visible spectral range as it blue-shifts with increasing span leading to a peripheral pink shade (in the areas with bigger angles in relation to the light source). In turn, the high degree of specularity in the NIR peak of *A. laetus* explains its metallic appearance in NIR images (Figure 2-4).

We used Principal Components Analysis (PCA) to visualise the diversity of spectral properties among the 35 specimens of Christmas beetles (30 species) (Figure 2-5; expanded results in **Figure A-7**, **Figure A-8**, **Table A-7** and **Table A-8**). The spectral properties of Christmas beetles do not cluster according to genus (Figure 2-5). Species of the genus *Anoplognathus*, in particular can achieve a great variety of combinations of the parameters we measured. In fact, even beetles from the same species, specifically colour morphs of *Repsimus manicatus*, *A. smaragdinus* and *A. porosus*, are distant from each other in the PCA. This great variability can be explained by the fact that multiple selective pressures may determine the overall optical effects arising from the beetle elytra. Optical effects are a combination of modular pigment and structural components (Stuart-Fox *et al.*, 2020; Shawkey & D'Alba, 2017). Each of these components may be influenced directly by selection for thermoregulation, communication or camouflage, as hypothesized for the scarab *Chrysina gloriosa* (Agez *et al.*, 2017), or indirectly by selection for mechanical resistance, anti-adhesive properties or friction reduction as shown in other beetle families (Seago *et al.*, 2009).

- **Correlation between spectral properties in Christmas beetles**

Our method enables exploration of the correlations between parameters for a given taxonomic group (Table 2-3). These correlations can reveal important information about potential structural or developmental constraints. For visible and NIR light, the presence of distinct peaks was associated with a shorter wavelength spectral location, high spectral purity, iridescence and specularity (this latter only in VIS). This is because short wavelength or UV-blue colours are generally produced by highly ordered nanostructures that generate peaked spectra with the characteristics mentioned above (Shawkey & D'Alba, 2017). Maximum direct reflectance is expected to be proportional to specularity because maximum reflectance is highest when all light is reflected at the specular angle (Osorio & Ham, 2002; Gruson *et al.*, 2019a) and our results for both NIR and VIS supported this expected correlation.

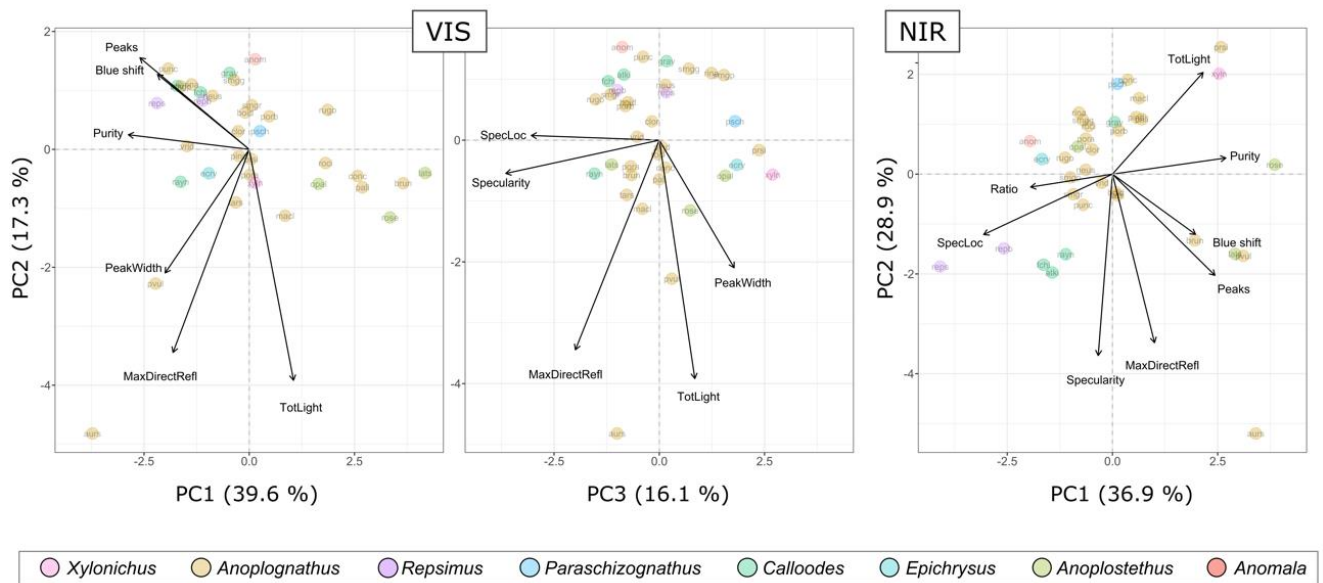


Figure 2-5. Multidimensional diversity of reflectance properties in Christmas beetles

Principal component analysis of the parameters used to describe visible (left and middle panels) and NIR (right panel) reflectance properties. Species do not group according to their genus or combinations of specific reflectance properties. Total hemispherical reflectance was abbreviated as “TotLight”, spectral location as “SpecLoc” and iridescence as “Blue shift”.

Some of our results differ from the expected patterns. For instance, broadband reflection has been associated with disorder in the structure (including irregularities in the surface and chirped or chaotic multilayers) (Stuart-Fox *et al.*, 2018; Johansen *et al.*, 2017; Cook & Amir, 2016; Campos-Fernández *et al.*, 2011; Shawkey *et al.*, 2009a). Therefore, it should be negatively correlated with specularity and iridescence (Moyroud *et al.*, 2017). However, metallic appearances (mirror-like with high distinctiveness of image; Franklin & Ospina-Rozo, 2021) combine a very broad band peak with high specularity and high maximum reflectance, as in *A. parvulus* and *A. aureus* (Figure 2-3), consistent with the phenomenon described for other gold beetles (Thomas *et al.*, 2007). In addition, beetles with an underlying broadband colour (low total reflectance influenced by absorption) combined with a smooth and clear overlying layer (Figure 2-2) may have a sheen, white gloss or contrast gloss effect (Franklin & Ospina-Rozo, 2021) and, therefore also high values of specularity and maximum directional reflectance. This explains the lack of negative correlation between peak width and specularity or iridescence, and why higher values of maximum directional reflectance (mirror angle) can correlate to wider peaks and high spectral purity. Overall, we did not find evidence for a strong correlation between iridescence and specularity (36% only). These properties are always strongly linked in simple, highly ordered or ideal structures (e.g. highly ordered thin films) but the correlation breaks down in biological materials due to their modularity, complexity and the presence of different types of disorder in the structure. The causality relations

between the nanostructures and optical effects in natural materials is an exciting and growing field and is promising to examine in Christmas beetles given the diversity of observed optical properties.

Total hemispherical reflectance seems to be independent of all other properties. However, we found an inverse correlation with the number of peaks for visible light, possibly because a broadband flat spectrum (light brown or white) has higher total reflected light than highly saturated peaks. Conversely, for NIR reflectance, we found a strong correlation between total hemispherical reflectance and shorter wavelength spectral location, higher spectral purity and low specularity. This may be due to the presence of multiple cases of sigmoidal NIR reflectance at wavelengths less than 1400 nm. This corresponds to the spectral power distribution of sunlight and would ensure reflection of a high proportion of the energy in sunlight if NIR reflection was used for thermoregulation. The NIR/VIS ratio correlates negatively with the total hemispherical reflectance in the NIR, suggesting that highly reflective beetles tend to have broad reflectance across both spectral ranges.

Table 2-3. Correlations between parameters that describe visible and NIR reflectance.

	Spectral Location		Peak Width		Total Hemispherical Reflectance		Spectral Purity		Blue-Shift		Specularity		Max. Directional Reflectance		NIR/VIS Ratio
	VIS	NIR	VIS	NIR	VIS	NIR	VIS	NIR	VIS	NIR	VIS	NIR	VIS	NIR	NIR
Number of Peaks	-0.34*	-0.47*	0.37*	NA	-0.39*	0.08	0.60*	0.53*	0.58*	0.54*	0.37*	0.31	0.2	0.58*	-0.27
Spectral Location			-0.32	NA	0.09	-0.71*	-0.71*	-0.77*	-0.16	-0.28	-0.13	0.35*	-0.16	-0.05	0.51*
Peak Width					0.07	NA	0.54*	NA	0.19	NA	0.1	NA	0.45*	NA	NA
Total Hemispherical Reflectance							-0.27	0.46*	-0.24	0.09	-0.26	-0.43*	0.25	-0.11	-0.36*
Spectral Purity									0.47*	0.33*	0.28	-0.13	0.32*	0.14	-0.09
Blue-Shift											0.36*	0.11	0.22	0.25	-0.36*
Specularity													0.62*	0.82*	0.08
Max. Directional Reflectance															-0.10

Note: Values correspond to the Pearson's correlation coefficients. * *p* value < 0.05. Iridescence is reported here as the magnitude of the blue-shift in spectral location. Abbreviation: NA, not assessed; NIR, near infrared; VIS, visual.

- Ecological and evolutionary implications

Visualising the multidimensional diversity of optical effects along with the correlations between different parameters can provide insights into whether certain optical properties tend to evolve together because of phylogenetic relationships, structural or developmental constraints or because they serve similar purposes. Some of the correlations we observed were weaker than expected from optical theory of ideal structures, highlighting the complexity and modularity of natural structures and suggesting that different parameters may be influenced by different selective pressures. For example, the iridescence and specularity in the visible range could be important for communication or camouflage (Kjernsmo *et al.*, 2020; Kjernsmo *et al.*, 2018; Kemp & Rutowski, 2007; Vukusic *et al.*, 2002; Schultz & Fincke, 2009), while the total hemispherical reflectance and NIR reflectance could be associated with thermoregulation (Bakken *et al.*, 1978; Medina *et al.*, 2018; Stuart-Fox *et al.*, 2017; Smith *et al.*, 2016a). To elucidate biological function, the diversity of optical properties observed in Christmas beetles need to be investigated in the context of the beetles' habitat and behaviour. Additionally, the great diversity of optical properties we observed in closely related species raises fascinating questions regarding the development and evolution of underlying mechanisms, such as whether they are evolutionarily labile or constrained by trade-offs (Maia *et al.*, 2016; Nördén *et al.*, 2021; Babarovic *et al.*, 2019; Lloyd & Nadeau, 2021).

Standardised methods, parameters and terminology will facilitate comparison between studies and species, while incorporating angle-dependency and NIR measurements will likely reveal new adaptations. Specular and diffuse reflectance and angle-dependency could be incorporated into models of animal colour vision (White, 2020; Simpson & McGraw, 2018; Endler & Mielke Jr, 2005) to ask questions about appearance to different receivers including conspecifics, predators and prey and how appearance changes depending on the geometry of illumination and viewing (Echeverri *et al.*, 2021; Simpson & McGraw, 2018). Currently, we have limited understanding of how iridescence and specularity are perceived and processed by different animals and whether they may have different biological functions, largely because of the previous lack of methods to study them separately (Stuart-Fox *et al.*, 2020; Doucet & Meadows, 2009; Morehouse & Rutowski, 2009). Our hope is that the approach discussed here to independently quantify angle-dependency of chromatic and achromatic components will stimulate research into their biological function. Finally, our results also revealed diversity in NIR directional reflectance, which raises questions regarding similarities and differences in the underlying mechanism among species and whether this diversity

represents adaptive variation. Further studies are required to understand the biological relevance of directional reflectance and NIR light manipulation.

2.5 | Data availability

In support of the Open Science Framework, find here a link and QR code to interactive web pages of my statistical analysis. This resource also provides access to the original data and permanent version tracking.

[Generalized approach code](#)



2.6 | CRediT Author Statement

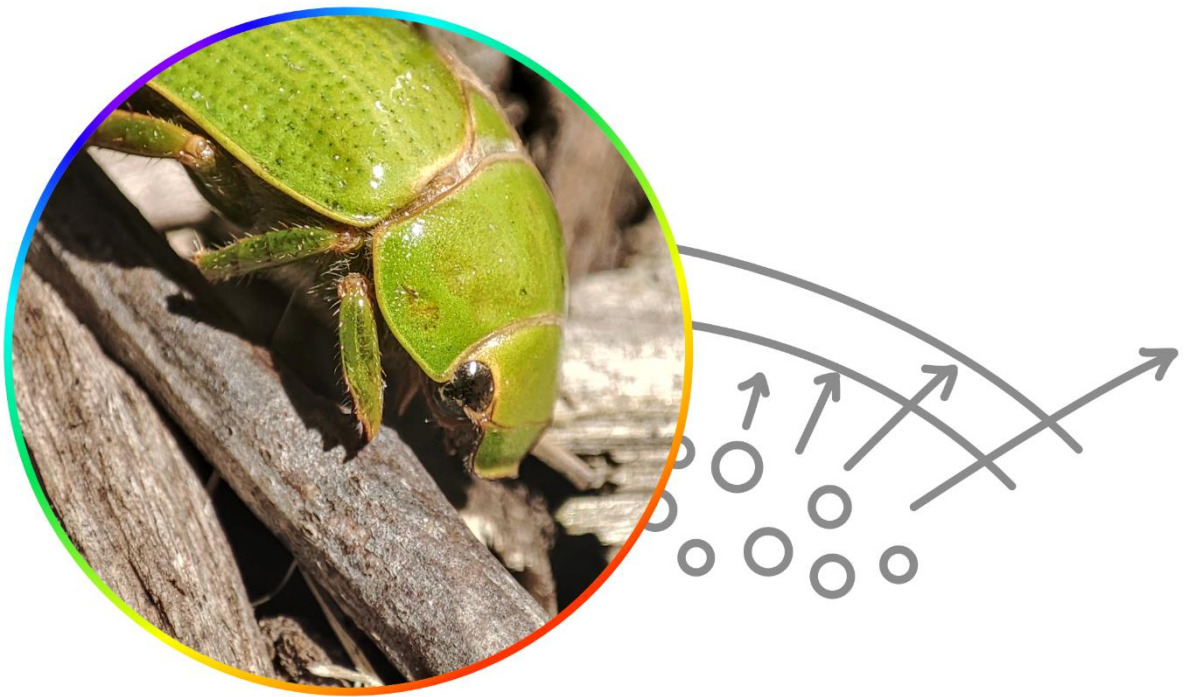
Laura Ospina-Rozo: conceptualisation, investigation, formal analysis, visualisation, and original draft. **Devi Stuart-fox:** conceptualisation, writing-review and editing, supervision. **Ann Roberts:** conceptualisation, writing-review and editing, and supervision.

2.7 | Acknowledgements

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Chapter 3 - Deconstructed Beetles

Bilayered composite materials produce green coloration with remarkably high near-infrared reflectance



This work has been revised and resubmitted following peer review in Materials Today Advances

3.1 Abstract

Beetle elytra (hardened forewings) are a promising source of inspiration to develop or enhance the performance of human-fabricated composite materials. The structures responsible for optical properties in the ultra-violet to visible spectrum (300 - 700 nm) have been extensively characterised, but we have limited knowledge of optical properties and their physical origin in the near-infrared (NIR; 700 - 1700 nm). We examined the elytra of three species of green scarab beetles (*Xylonychus eucalypti*, *Anoplognathus prasinus* and *Paraschizognathus olivaceus*) with very high NIR reflectance. We manually separated layers in the elytra to disambiguate their contributions to the overall optical response. We show that unlike other scarabs, nanostructures within the cuticle layer do not produce notable scattering. Instead, the cuticle resembles a pigment-based filter with 50% transmittance in the NIR and absorption in the visible spectrum contributing to the green appearance. Each species has a layer below the cuticle that appears white to the naked eye and produces broadband reflectance, particularly in the near-infrared; however, the structure of the white underlay differs markedly between the three species. In *A. prasinus* and *P. olivaceus*, the structure is disordered (no regular, repeated elements at optical length scales); whereas in *Xylonychus eucalypti*, the white underlay was notably thinner and comprised quasi-ordered hollow cylindrical structures embedded in a chitin matrix. We modelled the coherent scattering produced by this structure to demonstrate that it is responsible for broadband visible and NIR reflectance. We discuss biological implications and technological applications of the composite structure of beetle elytra.

3.2 Introduction

Composite materials combining different structural and functional properties are ubiquitous in nature and of substantial interest in materials science (Schroeder *et al.*, 2018). The mechanical properties of natural composite materials have been the subject of substantial interest (Rivera *et al.*, 2021), but the contributions of different layers to optical responses in different wavebands have received little attention. Most photonics studies have focused on structures that manipulate UV-visible light (UV-VIS: 300 - 700 nm) in natural materials such as butterfly wings, insect cuticles and bird feathers (Chen *et al.*, 2021b; Srinivasarao, 1999; Shawkey *et al.*, 2009a; Vukusic & Sambles, 2003), but very few have examined structures contributing to near infrared (NIR: 700-2500 nm) optical responses (chameleons: Teyssier *et al.*, 2015; Gonome *et al.*, 2018; birds: Stuart-Fox *et al.*, 2018). This is important for passive cooling (i.e. reducing absorption of radiative heat) because NIR wavelengths account for approximately half of the radiant energy in direct sunlight (Stuart-Fox *et al.*, 2017). Understanding the properties and architecture of the natural structures that interact with NIR light is important to understand the biological relevance of NIR reflectance and its potential for bioinspired applications.

In both animals and manufactured materials, passive cooling can be effectively achieved by using bilayer composite materials that reflect light differently in visible and NIR wavebands. For animals, visible colour is important for camouflage or signalling; while reflection of NIR light, which is largely invisible to most species, can aid thermoregulation (Stuart-Fox *et al.*, 2017). Similarly, passive radiative cooling is increasingly important to mitigate the temperature rise in transformed environments and reduce the costs and environmental impact of energy powered cooling systems (Chen *et al.*, 2020b; Mandal *et al.*, 2020; Chen *et al.*, 2021a; Mandal *et al.*, 2018). The traditional solution has been to chemically modify substances to increase their NIR reflectivity, however these chemicals are toxic or involve toxic components in their synthesis and they can also deteriorate over time (Rosati *et al.*, 2021). More recently, bilayer coatings have been developed to provide a more effective alternative. These bilayer coatings comprise a broadband reflective underlay to maximise scattering of NIR light and reduce solar heat gain; and an upper layer to filter some of the reflected wavelengths in the visible spectrum to produce the desired colour. This has been proposed as a scalable and effective solution to facilitate the cooling of buildings (Chen *et al.*, 2020b; Mandal *et al.*, 2020; Chen *et al.*, 2021a; Mandal *et al.*, 2018), which is

more effective and environmentally friendly than NIR reflective chemically altered pigments (Primo *et al.*, 2019; Rosati *et al.*, 2021).

Beetles are a promising source of inspiration to develop or enhance the performance of human-fabricated composite materials for passive cooling. Beetles are the most diverse and successful animal group on earth and a major focus of bioinspired materials design (Schroeder *et al.*, 2018). Key to the success of beetles in colonizing almost every environment on earth is the modification of the first pair of wings into a shield covering the body - the elytra (Stork, 2018; Lawrence & Newton, 1982). Beetle elytra are a natural composite material with different layers and air sacs to be able to simultaneously serve multiple functions, for example: light weight for flight, protection from mechanical damage (both strain and impact), hydrophobicity, self-assembly and many others (Neville, 2011). Thus, beetle elytra comprise modular and multipurpose structures to simultaneously accommodate multiple functions. The general architecture of beetle elytra is a sandwich structure (Characterisation of the transversal section of the cylinders in *Xylonychus eucalypti*'s white underlay.1A). The upper lamination (outermost dorsal surface) and lower lamination (ventral side of the elytra: surface facing the body of the beetle) are separated by a series of air sacs and trachea. The inner surface of the lower lamination contains a honey-comb array of walls. In the intersections of these walls vertical cylinders called trabeculae connect the upper and lower lamination (Van de Kamp & Hartmut, 2010; He *et al.*, 2015; Chen *et al.*, 2018). The upper lamination is mainly constituted by the cuticle, which has been the main focus of research because it contains diverse nanostructures that give rise to dazzling optical effects such as metallic, mirror-like and iridescent appearance (Seago *et al.*, 2009). These effects may be produced by one or more layers of the cuticle, known as epicuticle, exocuticle, and endocuticle (Seago *et al.*, 2009). Traditionally, it has been considered that the main role of the trachea, trabecula, fibres of the trabecula and the honeycomb structure is to provide structural support (He *et al.*, 2015; Chen *et al.*, 2018; Lomakin *et al.*, 2011; Neville, 2011; Rivera *et al.*, 2021). Thus, their contribution to the overall appearance of the elytra and NIR optical response remains unknown.

Optical effects from the beetle elytra are generally attributed to coherent scattering by very regular nanostructures, but given the diversity of beetle species, some species may have structures and mechanisms that remain unexplored. For example, beetles of the scarab family (Scarabidae) commonly have chitin fibres arranged in a helicoidal chiral structure (also known as Bouligand or plywood array) with optics analogous to a

cholesteric liquid crystal (**Figure B-2**)(Finlayson *et al.*, 2017; Sharma *et al.*, 2014). This helicoidal structure results in circular birefringence which produces optical activity (Arwin *et al.*, 2012); and mechanical fracture resistance (Zaheri *et al.*, 2018). These Bouligand structures are widespread in nature and are of significant interest to materials scientists (Mitov, 2017; Wang *et al.*, 2020; Boyon *et al.*, 2021); however, polarization (as a proxy for the presence of the chiral structures) is present in only 8 out of 15 subfamilies of Scarabeidae (Pye, 2010). Thus, it is possible that other scarabs have alternative mechanisms to manipulate light. Furthermore, in contrast to optical responses in the visible spectrum, the nanostructural architecture underlying the high diversity of NIR light manipulation in scarabs (Ospina-Rozo *et al.*, 2022b) remains a mystery. From previous observations in Christmas beetles, we noticed that some species of scarabs have a distinctive pale colour on the ventral side of the elytra, which can be seen with the unaided eye as a white underlay located in between the two laminations of the elytra (**Figure B-1**). We hypothesised that this layer could contribute to light scattering, particularly in the NIR.

We examined the elytra of three different beetle species from different genera of Scarabeidae family: *Xylonychus eucalypti*, *Anoplognathus prasinus* and *Paraschizognathus olivaceus*, which have a white underlay (**Figure 3-1A** and **Figure B-1**) and produce a green appearance and high NIR reflectance from the dorsal side. We described the composition of the elytra in the three species and evaluated the contributions of the cuticle and white underlay to the overall appearance of the elytra. Our results suggest that unlike other scarabs, the three species studied here have a cuticle that is largely transmissive and does not reflect light with nanostructures. Instead, the elytra comprise composite materials with the upper cuticle layer acting as a green filter overlying a layer that produces broadband reflectance, which is higher in the near-infrared. Structural analysis of the white underlay revealed quasi-ordered structures (i.e. partial absence of organisation in either spacing, size or spatial disposition (Prum *et al.*, 1999a; Prum & Torres, 2003b; Mouchet *et al.*, 2020)) in *Xylonychus eucalypti*, in contrast to structures that were highly disordered at different length scales in the other two species. As only the structure of the white underlay of *Xylonychus eucalypti* was amenable to optical modelling, we modelled the coherent scattering produced by the quasi-ordered structures in this species to examine whether they are responsible for broadband visible and NIR reflectance. We discuss how the optical responses of composite materials in beetle elytra may be an advantage in different biological contexts as well as a source of inspiration for passive cooling technologies.

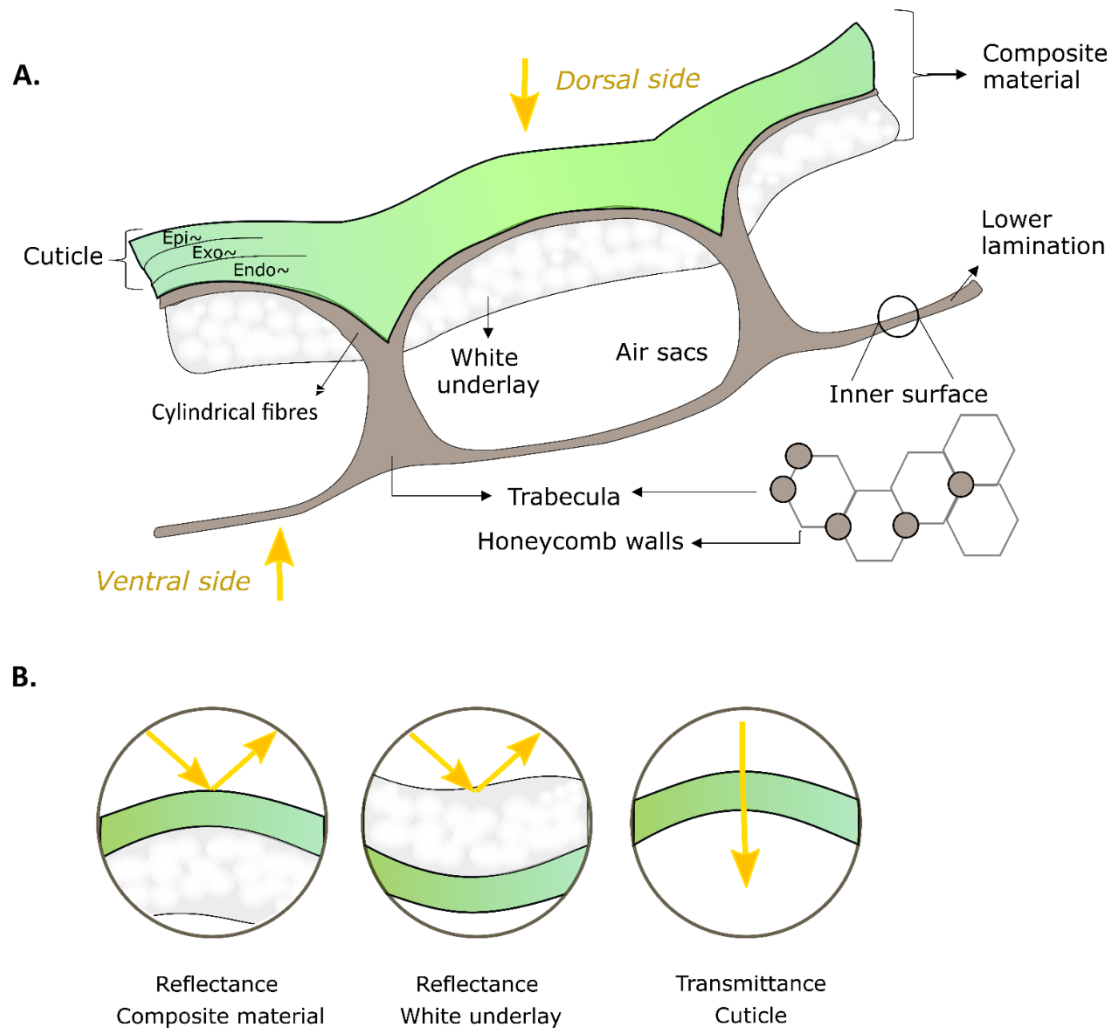


Figure 3-1. Schematic of the composite material of beetle elytra

A) In the studied beetle species, a white underlay is located immediately under the upper lamination, and these two elements constitute the composite material. The upper lamination is composed mainly by the cuticle which can be subdivided in epicuticle, exocuticle and endocuticle. The air sacs between laminations ensure the elytra are sufficiently light weight for flight (Iwamoto *et al.*, 2002). The ensemble of the honeycomb walls and trabecula confers mechanical stability to the elytra (Chen *et al.*, 2018). B) Examples of different configurations used to measure the optical properties of the two layers forming the composite material. Measuring reflectance from the dorsal side can be used to study the optical properties of the whole elytra or the intact composite (after removing the lower lamination). Measuring reflectance from the ventral side can be used to study the properties of the white underlay. Transmittance can be measured dorsally or ventrally. To study only the cuticle, we manually removed the white underlay.

3.3 Methods

3.3.1 | Beetle Elytra

The specimens of *Xylonychus eucalypti*, *Anoplognathus prasinus* and *Paraschizognathus olivaceus* were obtained from the Australian National Insect Collection (ANIC). We

removed the left elytron of each beetle to conduct destructive sampling. The three species studied here have a white underlay located in between the two laminations (**Figure 3-1A** and S1). We use the term white underlay as a proxy descriptor based on the visual appearance, although the origin and reflectance properties of this material may be different for the three species. This white underlay together with the upper lamination form the composite material of interest (**Figure 3-1A**). To study the composite material, we carefully removed the lower lamination of the elytra with a scalpel. Since the major component of the upper lamination is the cuticle, we use this term for brevity. We contrasted the properties and structure of the composite material with those of the cuticle with the white underlay removed. It is not possible to examine the white underlay in isolation because it cannot be removed from the cuticle without damaging it (**Figure 3-1B**).

3.3.2 | Spectral measurements

To measure the macroscopic sample reflectance (spot size 4 mm diameter), we obtained hemispherical reflectance spectra of the beetle elytra while still attached to the beetle body with an integrating sphere containing an inbuilt tungsten-halogen light source (400-2100 nm; ISP-REF; Ocean Optics Inc., Dunedin, FL, USA.) and 4 mm diameter sampling port connected to two spectrophotometers (Ocean Optics Inc., Dunedin, FL, USA), a USB 2000+ (400 - 1000 nm) and NIRQuest (1000 - 2100 nm) via a bifurcated optic fibre. Measurements were recorded using the software OceanView 1.6.7. and calibrated against a diffuse 99% reflectance spectralon standard (Labsphere, North Sutton, NH, USA).

To measure transmittance/direct transmission (Johnsen, 2012) we used a standard set up in which the spectrometers and light source are carefully aligned to send a beam of light through the sample and collect, on the opposite side, the portion of the beam that was neither absorbed nor scattered. For each measurement we placed one elytron between the light source and spectrometers. We used two light sources to include the UV-Visible range (PX-2 pulsed Xenon light) and the visible-NIR range (HL-2000 tungsten halogen light), combined via bifurcated optical fibres to illuminate the sample from the dorsal and ventral side of the elytra. To prevent saturation, we placed an 80% filter (SMA-ADP-031; Ocean Insight, USA) and a 0.4 neutral density filter (ND204B; Thorlabs Inc., USA) after the HL-2000 tungsten halogen light. We used the same two spectrometers connected to a bifurcated optic fibre to capture the light on the other

side of the sample. Measurements were recorded with the same software and calibrated against the reference (parallel beam of light without the sample).

To obtain macroscopic reflectance measurements of the different layers of the composite material (**Figure 3-1B**), we used the same spectrometers and light sources coupled to a goniometer. This allowed us to fix both the angle of incidence and collection to 15 degrees from the normal (Ospina-Rozo *et al.*, 2022b) and reduce the sampling area to a 1-mm diameter spot to focus on the areas of the elytra that were manually altered. We calibrated measurements against a diffuse 99% reflectance spectralon standard (Labsphere, North Sutton, NH, USA). As measurements were taken at the specular angle, it is possible to obtain values >100% reflectance relative to the diffuse standard but these values are not problematic since all samples were compared to the same reference (Ospina-Rozo *et al.*, 2022b); however the samples were primarily diffuse, so measurements of reflectance at the specular angle are a reasonable descriptor of the reflectance integrated across angles (Ospina-Rozo *et al.*, 2022b).

We measured the reflectance of the intact elytra (i.e. with the lower lamination), the white underlay (i.e. the composite material, measured from the ventral side) and the cuticle (i.e.; underlay removed; **Figure 3-1B**). We measured transmittance of the composite material (elytra without the lower lamination) and the cuticle. It is not possible to measure transmittance of the white underlay since it cannot be isolated from the other components without damaging it. The transmittance and reflectance profiles were analysed with the R package pavo (Maia *et al.*, 2019).

3.3.3 | SEM Microscopy

We used high resolution scanning electron microscopy (SEM) to explore the composition of the beetle elytra and to identify the structures that comprise the white underlay in the three species by comparing elytra with and without the white underlay. We used a sharp blade to cut transversal sections (perpendicular to the antero-posterior axis of the beetle) from the intact elytra and the cuticle (after removing the lower lamination and the white underlay) of the three species under a dissecting light microscope. Subsequently, the samples were mounted on independent stubs with carbon conductive tape and then sputter-coated with gold for 3 minutes using a QT5000 sputter coater (Quorum, United Kingdom). Finally, the samples were analysed using a SU7000 field emission scanning electron microscope (Hitachi, Japan) at 2.00 kV. In

order to determine whether the quasi-ordered nanostructures in the white underlay of *X. eucalypti* consisted of a series of spherical air bubbles or air cylinders, we repeated the same procedure with longitudinal sections of the elytra (parallel to the antero-posterior axis of the beetle). Finally, in order to extract the dimensions of different parts of the elytra, we analysed the SEM images of the three studied species with the basic measuring tools in Fiji, ImageJ (Schindelin *et al.*, 2012)

3.3.4 | Microscatterometry

We used microscatterometry to obtain angle-resolved spectral measurements of components of the composite materials of beetle elytra at the microscopic scale. The microscatterometry measurements were limited to the visible spectrum due to the wavelength limits of silicon-based technologies that form the basis of all optical microscopy. The set up consists of a highly focused broad band tungsten-halogen light source (Ocean Optics HL-2000) coupled to a microscope objective (Olympus, oil immersion (Leica, refractive index 1.518), 60x magnification, numerical aperture 1.42). The broad band source was collimated and expanded to fill the objective back aperture, thus illuminating the sample at angles from 0 to 69 degrees from the surface with a focal spot of approximately ~1 μm diameter. The light scattered by the sample was recovered by the same objective and an image of the objective back focal plane was projected onto a CCD camera (PIXIS 1024B, Princeton Instruments) via a spectrograph (Acton SP2300, 150 grooves/ml grating), see Schematic in **Figure B-3**. In this way, microscopic reflection spectra could be collected over an angle range from 0 to 69 degrees from the surface normal in a single shot (see **Figure 3-5**). Reflectance was measured with respect to an aluminium film reference. This technique has been used effectively to study light manipulation by structures in biological samples (Vukusic & Stavenga, 2009), and we used it here to explore the optical properties of each component of the composite material across multiples angles of incidence and reflectance. To study the intact composite material, we measured its reflectance dorsally. To study the white underlay, we measured the reflectance of the composite material ventrally. We selectively focused the illumination plane of the microscope objective on the white underlay ignoring the cuticle, since it is impossible to separate the white underlay from the cuticle without damaging it. Finally, to study the cuticle, we carefully removed the white underlay and measured the cuticle's reflectance and transmittance (**Figure 3-1B**). All samples were mounted on glass coverslips (170 microns thick) and immersed in objective immersion oil (Leica, refractive index 1.518) for refractive index matching to the coverslip/objective couple.

3.3.5 | TEM microscopy

Our measurements of the optical properties of the layers in the composite material showed that the white underlay in *Xylonychus eucalypti* is the most NIR reflective of the three studied species. Additionally, the SEM images showed that the white underlay of *A. prasinus* and *P. olivaceus* comprised highly disordered structures that are disordered at different length scales; whereas in *X. eucalypti*, the white underlay is much thinner in cross-section and comprises a quasi-ordered structure that may produce coherent scattering. To characterise these structures so that we could model the coherent scattering, we used transmission electron microscopy (TEM) to reconstruct a 3D model of *X. eucalypti* from transversal sections and to characterise the shape, spacing and orientation of the nanostructures. We did not perform TEM for *A. prasinus* and *P. olivaceus* due to the length scale of the structures in the underlay and because the structures are not amenable to analytical modelling. For TEM of *X. eucalypti*, we followed the standard procedure for block face imaging: we stained the samples with heavy metals, dehydrated them in subsequent dilutions of ethanol and acetone, and embedded them in Spurr's resin (ProSciTech) (Deerinck *et al.*, 2010; Lippens *et al.*, 2019). We obtained serial sections of 70 nm thickness up until completing the sectioning of 1 μm depth in the sample, with an EM UC7 ultramicrotome (Leica, Germany) and imaged them using a TecnaiTM Spirit transmission electron microscope (FEI - ThermoFisher, United States).

In order to determine the shape, orientation and separation of the particles in the white underlay of *X. eucalypti*, we sampled 5 rectangular regions of 6 x 4 μm of the white underlay from each of the TEM images. We transformed the TEM rectangles into a binary image showing dark electron-dense regions that represent the chitin matrix and white patches that represent the air space inside the cylinders. Then, we used the plug-in 'Analyze particles' in Fiji, ImageJ (Schindelin *et al.*, 2012) to automatically identify these white patches, also called regions of interest (ROI), number them and fit an ellipse to each of them. From this plug-in we obtained the following parameters for 738 ROI: x and y centroid (indicating the position of the ROI in the image), major and minor axis of the correspondent ellipse, aspect ratio (ratio between the major and minor axis), circularity (proportion between the area and perimeter of the ellipse) and angle between the primary axis and a line parallel to the x-axis of the image (indicating if the ellipses have a random orientation in the matrix). In addition, for 88 ROI we identified an adjacent ROI and calculated the centre-to-centre distance by calculating the Euclidean distance between their centroids. All statistical analyses were carried out in the software R (R Core Team, 2021). Finally, to produce a 3D reconstruction of

the structures in the white underlay of *X. eucalypti*, we aligned the consecutive transversal TEM sections and selected features that consistently repeated across images. These features were followed through the sections and assembled together using the software Midas - IMOD (Kremer *et al.*, 1996; Deerinck *et al.*, 2010; Lippens *et al.*, 2019).

3.3.6 | Computational modelling

We implemented computational models to investigate the physical origin of the optical response of the quasi-ordered structures in the highly NIR-reflective white underlay of *X. eucalypti*. We created a 2D photonic crystal model assuming the structure to be a chitin matrix with air cylinders aligned along the longitudinal axis of the elytron (here equivalent to the z plane) (Figure B-4). We implemented a 2D photonic crystal model because our SEM longitudinal images showed that the structures in the white underlay had a more cylindrical rather than spherical geometry (A). We analysed the interaction of the structure with light of different wavelengths and polarizations using the Finite Element Method (FEM) as implemented in COMSOL Multiphysics (5.6)®. We modelled the underlay as a hexagonal array of identical cylindrical air voids (refractive index, $n = 1$) with circular cross-sections embedded in a chitinous matrix (refractive index of $n = 1.5$). The wavelength-dependence of the index of refraction of chitin is well known (Leertouwer *et al.*, 2011; Azofeifa *et al.*, 2012), however we used a refractive index of 1.5 because this value is a good approximation to describe the interactions with visible and near-infrared light and we did not consider UV wavelengths (Azofeifa *et al.*, 2012). The array was assumed infinite in the transverse x-direction and included 5 rows of cylinders in the vertical y-direction. Periodic boundary conditions were applied on the sides, non-reflecting ports implemented on the excitation and output boundaries, and the wavelength was swept from 400 nm to 1700 nm. Plane waves were incident on the structure from the y axis and both z-polarization (electric field parallel to the axis of the cylinder, i.e. the antero-posterior axis of the elytra) and x-polarization (electric field perpendicular) excitation was considered. The output of these models are the simulated reflectance spectra. Since no absorption was assumed in the model, the transmission was given by the inverse of the reflectance.

We considered different scenarios based on the parameters obtained from analysis of TEM images. To examine the effect of the particle size we created three models assuming circular cylinders with a right section (transversal) of 602, 660 and 732 nm diameter and constant centre-to-centre distance of 868 nm. Given that the right

section of the cylinders in the white underlay was fitted to an ellipse (not a perfect circle) in our image analysis, we determined the diameter of the cylinders in our models as the average of the minor and major axis of the ellipses at the 25, 50 and 75% percentiles respectively in our measurements from TEM images (Table 3-2). To examine the effect of particle separation, we created two models assuming circles with 660 nm diameter and centre-to-centre distance of 732, 868 and 967 nm corresponding to the 25, 50 and 75% percentiles respectively in our TEM measurements (Table 3-2). To evaluate if the structure had polarization sensitivity, we compared the two polarizations x and z in all these models. Finally, to test the effect of different angles of incidence we compared two models with cylinders of 660 nm diameter at 868 nm centre-to-centre distance, and incident light coming from the normal or 30 degrees angle. Overall, this set of models enabled us to explore whether the structures in the white underlay of *X. eucalypti* produce coherent interference but incorporate enough variability to produce diffuse reflectance of visible and NIR light (consistent with our spectrometry data) instead of directional, specular and/or iridescent reflectance.

Finally, considering that the white underlay in *X. eucalypti* seems to contain a considerable degree of disorder, we also ran numerical simulations of the structure using a custom MATLAB script adapted from the algorithm previously used to study quasi-coherent scattering from similar structures with two distinctive refractive indices producing diffuse blue scattering in birds (Prum & Torres, 2003b; Prum *et al.*, 1999a) (Supplementary materials B8-Supplementary methods).

We did not model the white underlay of the remaining two species *A. prasinus* and *P. olivaceous* because the structures are not amenable to straightforward analytical modelling. Some disordered arrays such as the ones in the ultra-white scales of some beetles have been modelled as disordered multilayers, since a transversal section of the array reveals intercalating layers of tissue and air (Lafaita *et al.*, 2010; Luke *et al.*, 2010; Hallam *et al.*, 2009; Lee *et al.*, 2020). This approach is not appropriate for *A. prasinus* and *P. olivaceous* because the sheets and air cavities in the structures are greater than the relevant optical length scales ($\frac{1}{4} \lambda$ as expected for multilayers (Johnsen, 2012)). Similarly, although Mie scattering can predict the reflectance of disordered arrays (Mouchet *et al.*, 2020), the chitin sheets in the white underlay are flat and folded instead of spherical, and no exact solutions can be obtained using the T-matrix method for non-spherical scatterers (Mouchet *et al.*, 2020). This is an interesting problem and will be the subject of ongoing work.

3.4 Results

3.4.1 | Optical properties on the macroscopic scale

The three beetle species had similar reflectance profiles corresponding to green appearance and high NIR reflectance but different transmittance profiles (**Figure 3-2**). *P. olivaceus* had the lowest reflectance and highest transmittance, while *A. prasinus* had the highest reflectance and lowest transmittance (< 10%). Considering that the proportion of light that is neither reflected nor transmitted is equivalent to the light absorbed by the material (Johnsen, 2012), we also determined that the elytra of *A. prasinus* had the highest absorbance (Figure 3-2).

associated with the endocuticle and the epidermal cells, while the air sacs remained empty (**Figure 3-3**). In *A. prasinus* the white underlay was composed of a disordered cavernous structure formed by tightly packed chitin sheets that fold in a disordered manner creating microscopic cavities of air. For this species, the white underlay extended all the way between the upper and lower laminations filling the space that is usually empty for most scarab species. Tracheae appear embedded inside the white underlay (left panel in **Figure 3-3**). In *P. olivaceous* the white underlay also was formed by chitin sheets, but they were less packed and less folded. Instead, most of them were perpendicular to the laminations with opposite ends of the sheets often attaching to the upper and lower laminations. In both *A. prasinus* and *P. olivaceous* the sheets of the white underlay were highly corrugated (right panel in **Figure 3-3**). In *X. eucalypti*, the white underlay was notably thinner and did not contain corrugated sheets. Instead, it comprised hollow cylindrical structures embedded in a chitin matrix. The cylinders appear as an array of nanoscopic circles in a transversal SEM section (**Figure 3-3** central panel) and as long grooves in a longitudinal SEM section (**Figure 3-6A**). Although these cylinders were not perfectly aligned, they can be considered a quasi-ordered structure since they are relatively similar in size and spacing.

Overall, the SEM data showed that the cuticle is similar for the three species (bottom panel), but the structures of the white underlay differ greatly and have different degrees of disorder. The dimensions of the elytra and its subdivisions also differ for these three beetles (details in Table 3-1). In addition, these results highlight that our manual protocols to deconstruct the beetle elytra into its constitutive elements was effective, which was crucial for our interpretation of the measurements of optical properties in different configurations (as shown in **Figure 3-1B** and further explained below).

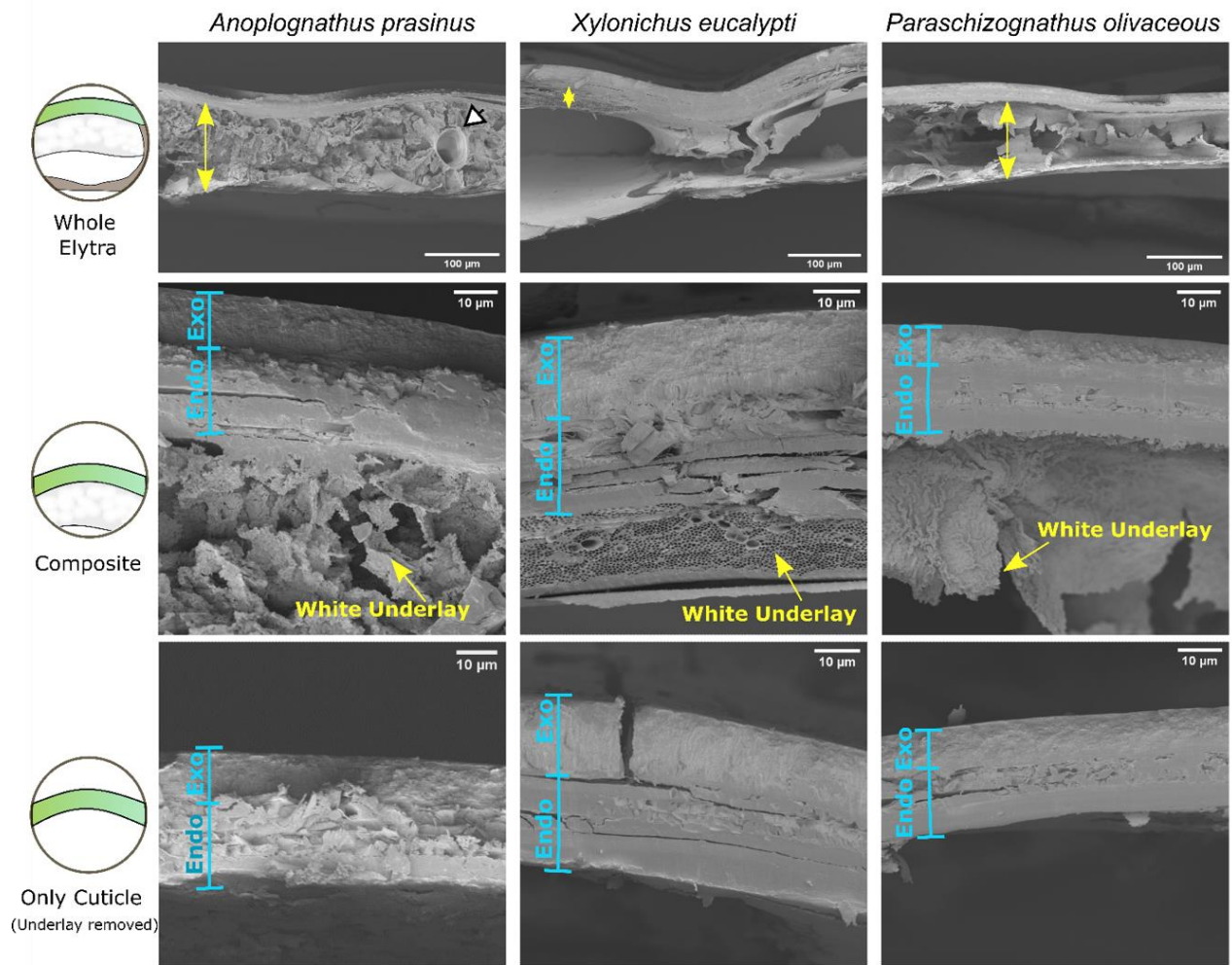


Figure 3-3. Architecture of the composite materials

Top row: SEM images of transversal sections of the elytra. Upper and lower laminations are visible as well as the air sacs in *X. eucalypti* and *P. olivaceous*, and the trachea in *A. prasinus* (white arrowhead). Vertical double-headed arrows highlight the differences in thickness of the white underlay for these three species. Middle row: Two subdivisions of the cuticle are shown: exo and endocuticle. The epicuticle is located dorsally to the exocuticle, but it is indistinguishable at this magnification. The structures of the material below the endocuticle are very different for these three species. Bottom row: the cuticle after manually removing the white underlay.

Table 3-1. Dimensions of different parts of the elytra in the three species

All measurements are given in μm and in the format mean $\pm$ sd considering measurements of three different SEM images per species. Images processed in FIJI ImageJ.

	<i>A. prasinus</i>	<i>X. eucalypti</i>	<i>P. olivaceous</i>
Elytra - Max. thickness	157.10 $\pm$ 1.51	218.65 $\pm$ 9.14	140.25 $\pm$ 8.09
Elytra - Min. thickness	134.67 $\pm$ 0.02	145.53 $\pm$ 0.56	95.64 $\pm$ 4.59
Air sacs - Av. thickness	closed	156.38 $\pm$ 25.56	86.26 $\pm$ 19.11
Trachea- Diameter	47.54 $\pm$ 7.86	Not seen	Not seen
Upper lamination- Thickness	33.72 $\pm$ 1.42	57.71 $\pm$ 1.19	22.82 $\pm$ 0.87
Exocuticle - Thickness	11.86 $\pm$ 0.33	14.43 $\pm$ 2.57	9.85 $\pm$ 1.32

3.4.3 | Optical properties of the different elements in the composite materials

In all three species, the cuticle had very low reflectance across the spectrum in comparison to the white underlay (Figure 3-4). We did not, in fact, obtain significant reflectance from the cuticle alone with microscatterometry. Additionally, the cuticle was transmissive in longer wavelengths $>700\text{nm}$ ($\sim 50\%$ of the light Figure 3-4), while it transmitted only a maximum of $\sim 30\%$ in wavelengths $< 700\text{nm}$ (Figure 3-4) with minima around 650nm (Figure 3-4 and Figure 3-5). These minima are accentuated in the reflectance profile of the whole elytra (in comparison to the cuticle alone, Figure 3-4). Considering the low reflectivity of the cuticle, the transmittance minima are likely due to absorption attributed to the presence of pigment and consequently could be associated with a green appearance. The *Xylonicus eucalypti* cuticle had one additional high energy minimum potentially due to a pigment absorption vibrational replica (Figure 3-5).

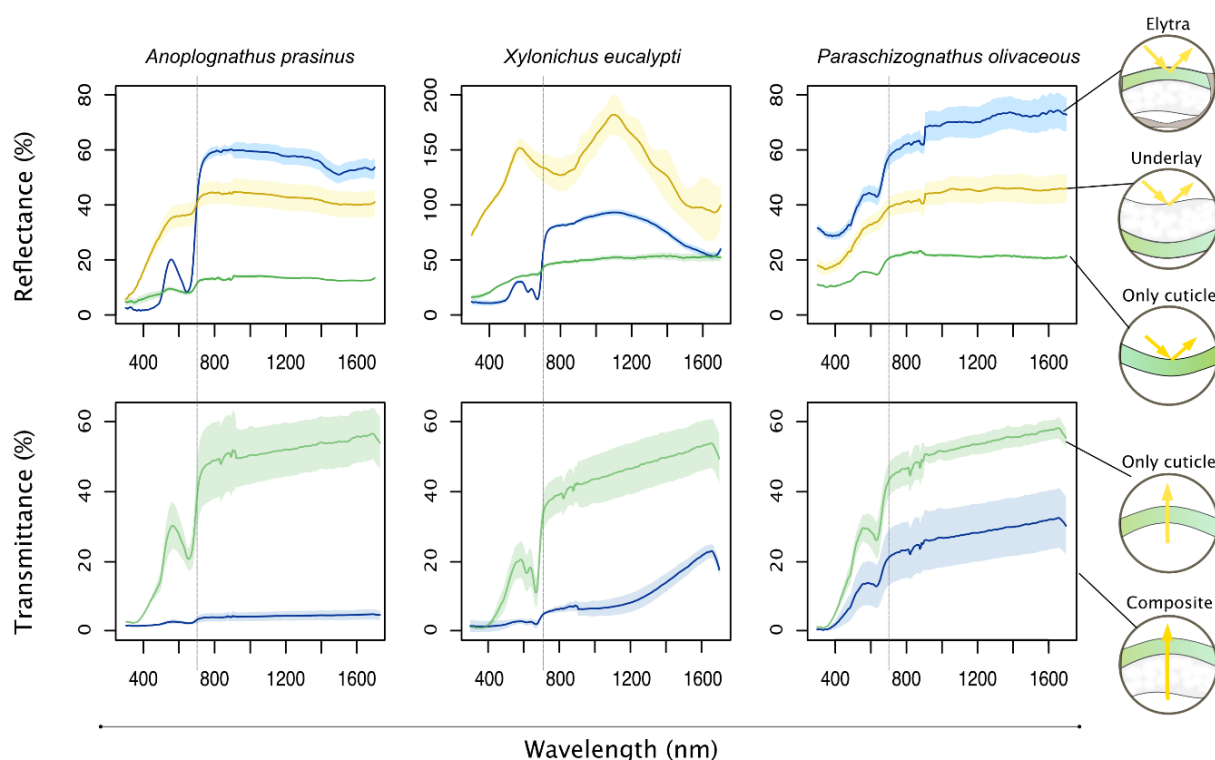


Figure 3-4. Optical properties of the different elements in the composite material at the macro scale

Reflectance was measured at the specular angle. For the three species, the “white underlay” is a broadband visible and NIR reflector (gray vertical lines at 700 nm), while the isolated cuticle has lower reflectance across the spectrum. *X. eucalypti* is the species with the most reflective white underlay (note the scale in the y axis). The reflectance on the dorsal side of the elytra differs from the reflectance of the white underlay. The cuticle is more transmissive than the intact composite (cuticle + white underlay).

In contrast to the transmissive cuticle, the white underlay of all three species had high broadband reflectance, which was notably higher in the NIR (>700nm) than in the visible spectrum (**Figure 3-4**). The white underlay also reflected light diffusely across a wide range of angles (up until 900 nm in **Figure 3-5**) as expected for these species (Ospina-Rozo *et al.*, 2022b). The reflectance spectra of the white underlay in *A. prasinus* and *P. olivaceus* are similar with increasing reflectance in the longer visible wavelengths and a homogeneous flat reflectance beyond 900 nm around 40%, while the reflectance spectra of *X. eucalypti* had two broad maxima (peaks) with reflectance higher than the other two species across the full spectrum (>100%, i.e., even greater than the Lambertian surface of the white standard) (**Figure 3-4**). The microscatterometry results also showed that the white underlay of *X. eucalypti* was the most reflective of the three species in the visible range (**Figure 3-5**). The reflectance of *P. olivaceus* appeared to be much weaker when measured with microscatterometry (**Figure 3-5**) than with spectrometry (**Figure 3-4**). The elytra (with the lower lamination) seem more reflective than the white underlay for *A. prasinus*

and *P. olivaceous* in the spectral data (Figure 3-4) but not in the microscatterometry data (Figure 3-5). This could be because for these two species, the white underlay may suffer small alterations after the removal of the lower lamination potentially causing underestimation of the white underlay reflectance apparent only at the macro level.

The composite material (cuticle + white underlay) measured from the ventral side (white underlay facing the light source) also varied in transmittance between species. Low transmittance of the composite in this configuration is expected for *X. eucalypti* (< 10%) since most of the light is reflected by the white underlay, therefore it cannot be transmitted. Remarkably, the white underlay of *A. prasinus* and *P. olivaceous* have a similar reflectance, but the composite material in *P. olivaceous* has high transmittance (reaching approx. 70 % around 580nm and no less than 20% in the NIR), while the composite material in *A. prasinus* has the lowest transmittance of the three species (<5% across the full spectrum). This could be explained by high absorption in the white underlay of *A. prasinus*.

The microscatterometry shows enhanced reflectance at high angles (>40 degrees from the surface normal) for all species, an effect commonly associated with total internal reflection. In this phenomenon, the incident light beam is completely reflected with no attenuation because it travels from a medium of higher refractive index onto a medium of lower refractive index at an angle greater than or equal to the critical angle (Johnsen, 2012). Given that the beetle elytron has microstructures able to repel water and other substances (Sun *et al.*, 2012), it is likely that the refractive index-matching oil in which the samples are immersed does not fully infiltrate the space in between the two laminations of the elytra. Thus, the enhanced reflection at larger angles could be produced by the interfaces between chitin/immersion oil (refractive index = 1.5) and air pockets inside the white underlay (refractive index = 1) for which the critical angle is 41 degrees. However, disordered structures in other beetles can favour enhanced reflectance at larger angles for other reasons (Lee *et al.*, 2020). The asymmetry in reflectance at positive and negative angles from the normal could be a product of irregularities in the sample. Both the white underlay and the surface of the elytra contained irregularities at different scales and directions (**Figure B-5**). Even after ensuring the best focusing of the light beam, the target surface may not be flat, further contributing to the asymmetry in reflectance. We used polarised filters to collect the light with electric field perpendicular to the plane of incidence (s-polarisation) and with electric field in the plane of incidence (p-polarisation) for different alignments of the elytron. In Figure 3-5, we show the light reflected by the

structures only in s-polarized since we did not observe any obvious polarization events. This suggests that disorder in the nanostructures is enough to cancel any such polarization effects, or else the polarization sensitivity of the structure is too subtle to be detected by our set up.

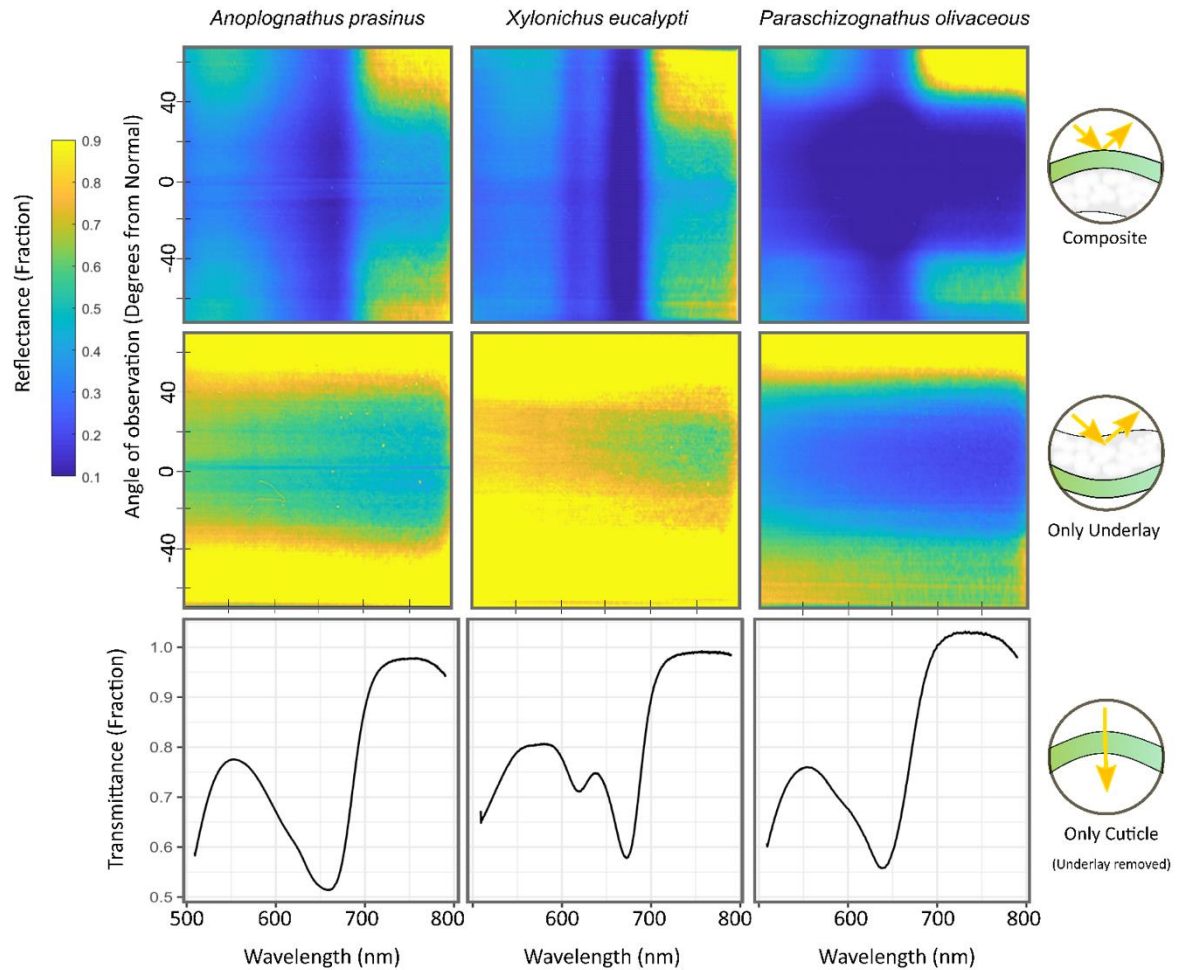


Figure 3-5. Reflectance in multiple angles and transmittance

Microscatterometry results. The samples were illuminated with unpolarized light, but the collected light was filtered for the TE-polarization (electric field perpendicular to the plane of incidence of the light over the filter). For the three scarabs, the diffuse reflectance pattern as a function of angle produced by the composite material (top) is the result of the additive interaction between the underlay reflectance (middle) and cuticle transmittance (bottom), in other words between the structural and pigment-based component in the composite material. The transmittance spectra of the cuticle of the three beetles are remarkably similar, though a vibrational replica could be resolved in the pigment absorption in *X. eucalypti*.

3.4.4 | Structure of the white underlay in *Xyloniichus eucalypti*

As the white underlay of *Xyloniichus eucalypti* comprised quasi-ordered structures at optical length scales, which could produce coherent scattering, we conducted a more in-depth structural analysis using TEM to parameterise computational models. The TEM

images revealed considerable variability in the size, separation and orientation of the cylinders (Table 3-2). From the TEM image processing we found that the right section (transversal section) of the cylinders in the white underlay of *X. eucalypti* consists of ellipses with a fairly regular perimeter (circularity). The major axis of the ellipses varied between 603 and 1027 nm and minor axis between 290 and 646 nm (5% and 95% percentiles). We did not find any correlation between the separation of the ellipses and their size, i.e., the centre-to-centre distance was neither correlated with the ellipses major axis (Pearson's correlation $R=0.062$, $p\text{-value}=0.567$, C.I. $-0.151; 0.271$), nor the minor axis (Pearson's correlation $R=-0.12$, $p\text{-value}=0.259$, C.I. $-0.326; 0.091$). The major axis of the ellipses roughly aligns with the surface of the cuticle and the ventral surface of the elytra but it varies between -30 and 30 degrees (**Figure B-6**). The variability in the angles of the cylinders and in the thickness of the chitin matrix can explain the lack of correlation between the size of cylinders and their separation. From the 3D reconstruction we conducted based on sequential TEM images and the longitudinal SEM images (**Figure 3-5A**) we also observed that the cylinders do not align perfectly parallel to each other's axis. Instead, they zigzag and bend slightly adding disorder to the structure.

Table 3-2. Characterisation of the transversal section of the cylinders in *Xylonychus eucalypti*'s white underlay.

Parameter	Mean	sd	Percentiles		n
			25 %	75 %	
Ellipse major axis	807.4	168.62	741.25	883	738
Ellipse minor axis	510.87	109.37	462	581	738
Aspect ratio	1.63	0.46	1.34	1.8	738
Centre-Centre distance	867.69	200.47	732.28	966.54	88
Circularity	0.83	0.11	0.79	0.9	738
Angle	20.85	14.86	10.04	29.06	737

3.4.5 | Computational models

According to our models (**Figure 3-6B-E**) the two factors explaining the broadband reflection and high NIR reflection in *X. eucalypti* are the high variability in spacing between cylinders and the response of the structure to incident light from different angles. A change from the 25% percentile to 75% percentile in the size of the cylinders causes only a subtle change in the total reflectance (**Figure 3-6B**), while an equivalent

change in the spacing of the cylinders causes a significant shift in the reflected wavelengths (**Figure 3-6C**). Not surprisingly the modelled structure has different responses for the two polarizations (**Figure 3-6D**). This result contrasts with our experimental measurements that showed no polarization sensitivity, which could be because the cylinders do not perfectly align along a common z axis as shown by our morphological analysis and 3D reconstruction (**Figure 3-6A**). Finally, the incident angle has a considerable effect on reflectance (**Figure 3-6E**). At larger angles away from the normal, more NIR light is reflected.

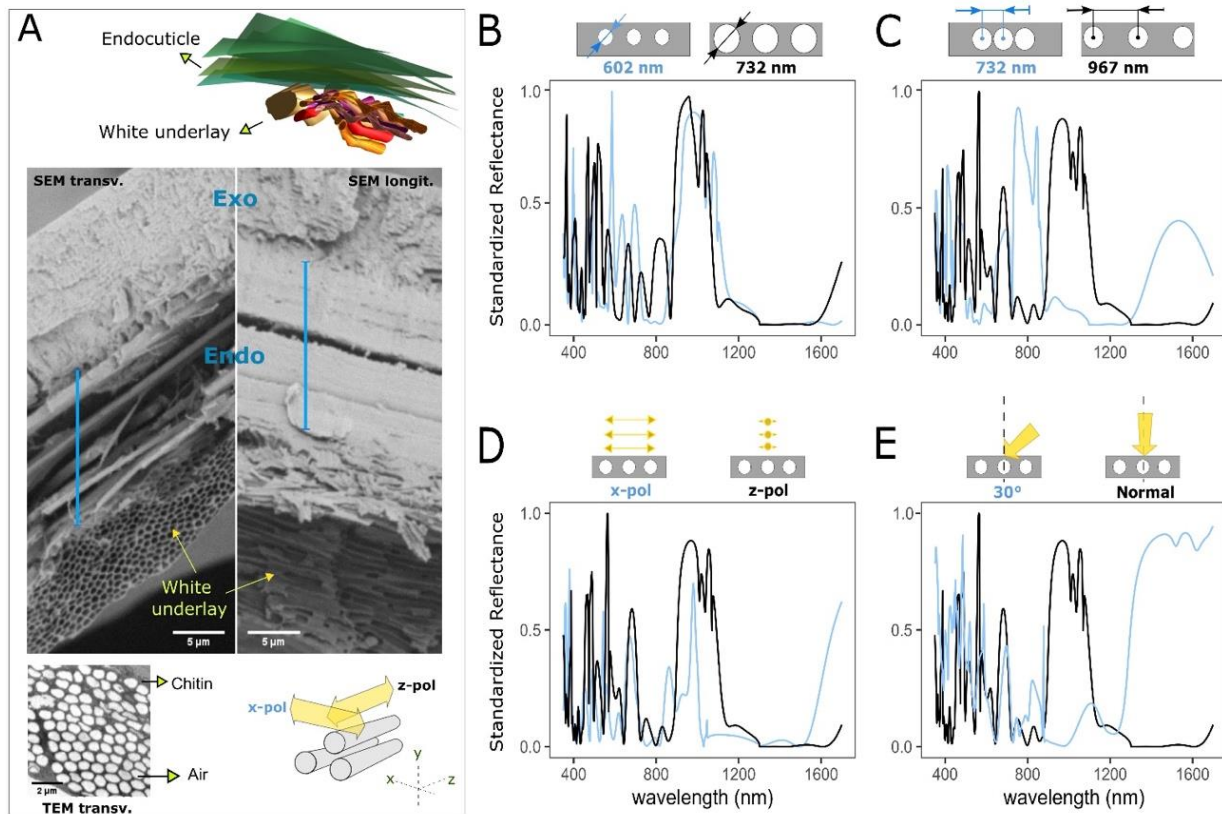


Figure 3-6. Modelling of the white underlay of *Xylonychus eucalypti*

A. We produced a 3D reconstruction of the structures in the white underlay by following the white circles (air spaces in the chitin matrix) through subsequent transversal TEM sections. We confirmed that these circles correspond to the transversal section of cylinders with longitudinal SEM sections. Thus, we modelled the white underlay as cylinders interacting with light in two polarizations: with its electric field parallel to the cylinder axis (z-pol) and perpendicular to the cylinder axis (x-pol). **B.** Effect of the size of the cylinders: comparing particles with different diameters (constant 868 nm centre-to-centre distance, normal incidence, z-pol). **C.** Effect of the separation between the cylinders: comparing different centre-to-centre distances (fixed 660 nm diameter, normal incidence, z-pol). **D.** Effect of the polarization of the incoming light (660 nm diameter and 868 nm centre-to-centre distance). **E.** Effect of the angle of incidence of the incoming light (660 nm diameter and 868 nm centre-to-centre distance, z-pol). SEM images in A showed that the cylinders are not perfectly ordered (quasi-ordered), which suggests that the overall reflectance of the structure is the sum of variations of the scenarios in B to E.

Our models are simplified scenarios that combine and overlap in the natural white underlay in *Xylonychus eucalypti*. For instance, we showed that the reflected wavelengths depend on the spacing between the cylinders. The spacing between the cylinders, in turn, varies independently from their size (**Figure B-6C**). Thus, the presence of cylinders with variable spacing could produce different reflectance peaks which on average determine the broadband reflectance associated with white appearance and high NIR reflectance (consistent with results in **Figure 3-4** and **Figure 3-5** for the white underlay). We showed that the modelled structure responded differently according to the angle of incidence of the light and polarizations. Although our models suggest a highly regular structure with parallel cylinders will have polarization sensitivity, our SEM images showed that the cylinders in the white underlay are not perfectly parallel to each other (**Figure 3-6A**). Disorder and misalignments are common in natural structures, and it generally reduced angle and polarization-dependent reflectance (Mouchet *et al.*, 2020; Zhao *et al.*, 2011), which explains why we did not observe polarization sensitivity in the scatterometry measurements. The overall broadband reflection with two apparent peaks generated by the white underlay (**Figure 3-4**) is likely the average of different responses of the structure across different angles and polarizations (**Figure B-7**), and overall broadband reflection as observed in our microscatterometry and spectroscopy measurements (**Figure 3-2** and **Figure 3-3**). In short, the photonic crystal model and morphological analysis explain the broadband diffuse reflectance and high NIR reflectance by the white underlay in *Xylonychus eucalypti*.

The quasi-ordered matrix model (Prum & Torres, 2003b) does not explain the reflectance of short wavelengths of less than 1000 nm (visible range) (**Figure B-8**) because the algorithm does not consider the polarization parallel to the axis of the cylinders, here called z polarization. Thus, in materials with cylinders like the white underlay of *X. eucalypti*, the reflected spectrum predicted by the adaptation of Prum and Torres's (2003) model corresponds only to the x polarization, i.e. when the electric field is perpendicular to the axis of the cylinders.

3.5 Discussion

In this study we demonstrated that contrary to other scarabs (Sharma *et al.*, 2014), the characteristic green appearance and high NIR reflectance of *Xylonychus eucalypti*, *Anoplognathus prasinus* and *Paraschizognathus olivaceus* is not produced by

nanostructures in their cuticle. Instead, they combine optical effects produced by different layers of the composite structure of the elytra to create the overall optical response in the visible and NIR spectrum. The composite material in the elytra of three species comprises the cuticle and a white underlay. The underlay generates broadband diffuse reflection particularly high in wavelengths >500 nm, while the cuticle resembles a conventional pigment-based filter in the visible spectrum (green appearance), with a strong absorption dip around 560nm. Incident light that is not absorbed by the cuticle is reflected by the white underlay and transmitted through the cuticle a second time. Thus, the combined effects of the white underlay and cuticle explain the overall green appearance with high NIR reflectance. Despite the broad similarities between species, they differed considerably in the optical properties and corresponding architecture and nanostructures of the white underlay. Most notably, in *Xylonychus eucalypti*, the white underlay had much higher reflectance, particularly in the NIR, and was also much thinner, comprising hollow cylindrical structures aligned parallel to the surface and embedded in a chitin matrix. Our optical models of this quasi-ordered structure support the conclusion that they are responsible for broadband scattering and very high NIR reflectance in this species. Variation in the spacing and orientation of the cylinders, together with integration over multiple angles of incidence, appear to be key to the broadband scattering. By contrast, in *A. prasinus* and *P. olivaceus*, the structure of the white underlay is disordered, comprising chitin sheets with different structural dimensions and degrees of packing and folding in the two species. Although the structures are not amenable to optical modelling, we can infer that they are responsible for the observed broadband scattering based on our disambiguation of the optical response of the layers of the composite material. Overall, our results suggest that the composite materials in these beetles have characteristics analogous to coloured bilayer coatings, but broadband VIS and NIR reflectance of the underlay is produced by different structures in the three species. Additionally, considering that these three beetles are not sister species, it is possible that the bilayered structures arose due to convergent evolution in response to similar selection pressures, rather than due to common ancestry.

The developmental origins of both the white underlay and the cuticle may provide insight into why optical structures in *A. prasinus*, *X. eucalypti* and *P. olivaceus* differ markedly from other closely related scarabs, which appear to lack the white underlay. For example, it is notable that the white underlay is formed by structures other than the cuticle. Given that the irregularly folded sheets forming the white underlay of *P. olivaceus* and *A. prasinus* are in the space between the two laminations, they may

come from the honeycomb, trabeculae or even the trachea (Iwamoto *et al.*, 2002). The white underlay in *X. eucalypti* is located in the region immediately below the endocuticle and not in the air sacs, so it may come from the cylindrical fibres of the trabecula. These components in the elytra have traditionally been associated only with mechanical support (Chen *et al.*, 2018) but we have shown that they also determine the optical properties of the elytra in some scarab species. Moreover, the insect tracheae contain spiral folds with corrugated surface called taenidia that function as structural support to prevent them from collapsing inwards (Harrison, 2009). The taenidia contain proteins and chitin fibres but lack cuticulin, which gives them a white appearance (Wigglesworth, 1990). Tracheae and tracheolae increase light reflectance in other insect structures such as the tracheal tapetum (Meyer-Rochow & Horridge, 1975; Dacke *et al.*, 2003; Gokan, 1982). The origin of the cuticle is also important. In the leaf beetle *Gastrophysa viridula*, a thin outermost green layer (absorption between 530 - 550 nm) is retained from the larva in the imago (adult immediately after ecdysis) but soon replaced by nanostructures (Onelli *et al.*, 2017). It could be interesting to explore if the composite materials of the elytra in the studied species retain elements from the larva. Studies examining the development of the elements in composite materials of various species as well as its frequency among scarabs, could be combined with ecological and phylogenetic data, to facilitate insight into its evolutionary origin and adaptive value.

The structural component of the composite material – the white underlay – strongly determines the differences in total transmittance, absorbance and reflectance of the whole elytra of the studied species. For example, densely packed disordered arrays such as the one in *A. prasinus* are effective at producing high broad band reflection (Chen *et al.*, 2020b; Johansen *et al.*, 2017) in beetles (Wilts *et al.*, 2018; Burg *et al.*, 2020; Burresi *et al.*, 2014; Luke *et al.*, 2010), birds feathers (Stuart-Fox *et al.*, 2018) and plant mesophyll (Slaton *et al.*, 2001). Although we did not attempt to model the white underlay of *A. prasinus* and *P. olivaceous*, we hypothesize that the chitinous sheets reflect light due to the difference in refractive indices between the air and chitin (like in plants (Gausman *et al.*, 1969)). The folding of these sheets creates air cavities, and the ridges and wrinkles increase their surface and disorder producing multiple scattering events. This idea is supported by the fact that *A. prasinus*, with densely packed chitin sheets in its white underlay has higher broadband reflectance than *P. olivaceous*. In addition, each time light is reflected by a surface, a proportion of that light is absorbed - structural absorption (McCoy *et al.*, 2018; McCoy & Prum, 2019; Shanks *et al.*, 2015), which could also explain why the thick white underlay from

A. prasinus has the lowest transmittance of the three species. According to our models the quasi-ordered array in *X. eucalypti* formed by air cylinders in a matrix of chitin produces high broadband reflectance by slightly varying the size, spacing and spatial alignment (Zhang *et al.*, 2022) of the cylinders in the chitin matrix. The reason for the lower transmittance in this species could be that most of the light is reflected, but it could also depend on the absorption coefficient (determined by the imaginary part of the refractive index (Johnsen, 2012)) of the chitin forming the array. Understanding how beetles can achieve different combinations of transmittance, reflectance and absorbance in their elytra is key to investigate their biological role.

The structural component of the composite material determines the wavelengths reflected and the absence of angle dependent effects. For example, our results showed that the cylinders in the white underlay of *X. eucalypti* can be modelled as an array of photonic crystals with enough variation in spacing and orientation to produce broad band diffuse reflectance (Hallam *et al.*, 2009). Unlike the highly ordered and aligned Bouligand structures present in other scarabs, this mechanism does not produce saturated peaks, iridescent reflectance, specular effects or circular polarization. Instead, the disorder in the structure results in enhanced white reflection as observed in other natural structures (Jordan *et al.*, 2014; Gur *et al.*, 2017). Worthy of consideration is the correlation between NIR and VIS reflectance. Due to the strong wavelength dependence of scattering (Johnsen, 2012), the nanostructures able to reflect NIR are likely to be relatively large and to interact with long wavelengths in the visible spectrum (red) and NIR. However, this is true considering only inter-scatterer correlations, i.e., the disposition of the particles in an array. If instead, the scattering behaviour of individual particles is also considered, secondary peaks of short wavelengths arise simultaneously (Magkiriadou *et al.*, 2014). This phenomenon combined with variability in the structures could produce multiple peaks in the NIR and VIS spectrum, which in turn explains why structures that reflect NIR tend to also be white (Stuart-Fox *et al.*, 2018) limiting the possibility of producing independent optical effects in the VIS and NIR wavebands with the same structure but increasing diffuse total reflectance.

The component of the composite material analogous to a pigment-based filter has similar optical properties and structure in the three studied species. *A. prasinus* and *P. olivaceous* belong to different genera of the subfamily Rutelinae, while *X. eucalypti* belongs to the subfamily Melolonthinae. Yet, all three species have very thin cuticles (here considered synonymous with the upper lamination) between 22 and 57 nm, and

the transmittance profile suggests that they contain similar pigment nanoparticles possibly less than 50 nm diameter (Chen *et al.*, 2020b). The origins of these green cuticles are intriguing. It is possible to obtain filtering materials with structures such as human-made resonating cavities and plasmonic surfaces (Yu *et al.*, 2014) but our electron microscopy images did not show evidence of structures like these in the studied cuticles. Thus, we believe that the filtering properties may be due to the presence of chemical compounds in the protein matrix around the chitin fibres. The most common pigments observed in beetles are melanins, ommochromes, carotenoids and flavonoids (Futahashi & Osanai-Futahashi, 2021). In the leaf beetle *Gastrophysa viridula*, it has been proposed that a thin outermost green layer (absorption between 530 - 550 nm) contains quinones and melanin (Onelli *et al.*, 2017). Another possibility is that the green colour is produced by mixtures of blue tetrapyrroles (bile pigments) and yellow plant-derived carotenoids as in other plant eating insects (Futahashi & Osanai-Futahashi, 2021; Shamim *et al.*, 2014). Further studies are needed to confirm the presence and absorption profile of the pigments in the beetle cuticle. Afterwards, comparison between different green scarab species could determine if their absorption profile varies according to the environment in the chitin matrix, and if the pigments of different species have independent evolutionary and/or developmental origins.

Given that this is the first observation of a broadband reflective white underlay in the beetle elytra, we can only hypothesise its biological function. The presence of a pigment-based filter overlying a white NIR reflective structure is an elegant solution to allow reflectance in different wavebands lacking in a single structure. This may be an adaptation to different selective pressures. For example, an untested hypothesis for the broad band scattering produced by some scarabs in the subfamily Melolonthinae (Burrese *et al.*, 2014; Wilts *et al.*, 2018) is that it is useful for camouflage against local white fungi. A non-mutually exclusive hypothesis is that broad band reflection extending to the NIR can aid in passive thermoregulation (Xie *et al.*, 2019; Stuart-Fox *et al.*, 2018; Zhang *et al.*, 2022). The species studied in this experiment may exploit the versatility of composite materials for passive thermoregulation (maximizing the reflected light and reducing the amount of radiative energy transferred to the beetle body) while maintaining a green appearance favourable for camouflage against a green background, e.g., a forest or any other habitat rich in vegetation. Conversely, some species of insects benefit from a light trapping mechanism to increase their temperature efficiently until they achieve their optimal temperatures (Shanks *et al.*, 2015) and this could be the case for beetle if there is heat transfer between the elytra and body. The air trapped in the densely packed structure such as the one in *A.*

prasinus underlay could also favour thermal insulation, but measurements of emissivity of the beetle body and elytra would be needed to support this hypothesis. The absence of air sacs in the elytra of some species may also have specific mechanical functions such as providing extra resistance to fracture or collapse of the elytra (Chen *et al.*, 2020a) or cushioning to reduce the energy transfer to the beetle body and internal organs (Kundanati *et al.*, 2018). However, beetles with larger air sacs are more likely to rely on flight since these adaptations facilitate the demands of aerial locomotion (Rivera *et al.*, 2021). Furthermore, natural composite materials are invariably multifunctional and it is possible that more than one selective pressure has driven the evolution of the white underlay in these scarabs. Ecological habits, microhabitat data and behavioural experiments are needed to better understand the biological relevance of the composite structure of the elytra for each species

Studying natural composite materials and their biological significance has the potential to inform passive cooling technologies. Natural materials combine characteristics that allow them to outperform human-fabricated technologies; they can absorb specific wavelengths in the visible spectrum while still allowing more than 80% NIR reflectance (Chen *et al.*, 2020b). This is a desirable feature in technologies such as coatings to control the temperature of buildings by minimizing radiative heat transferred to the building while simultaneously achieving the desired colour (Chen *et al.*, 2020b). In addition, air pockets in a composite material are useful as barrier materials for food packing (Yu *et al.*, 2020) or to enhance thermal insulation in textiles (Sun *et al.*, 2021) and even increasing fire resistance in light weight cement to implement in construction (Adhikary *et al.*, 2021). Further research could focus on comparing the effectiveness and scalability of different geometries of the scatterers and the self-assemble properties of natural composite materials.

3.6 Data availability

In support of the Open Science Framework, find here a link and QR code to interactive web pages of my statistical analysis. This resource also provides access to the original data and permanent version tracking.

[Deconstructed Beetles code \(QR code in next page\)](#)



3.7 CRediT Author Statement

Laura Ospina-Rozo: conceptualisation, investigation, formal analysis, visualisation and original draft. **Niken Priscilla:** software. **James Hutchison:** methodology, investigation, writing- review and editing. **Allison van de Meene:** methodology, investigation, writing- review and editing. **Nicholas W. Roberts:** conceptualisation, writing- review and editing. **Devi Stuart-fox:** conceptualisation, writing- review and editing, supervision. **Ann Roberts:** conceptualisation, software, writing- review and editing and supervision.

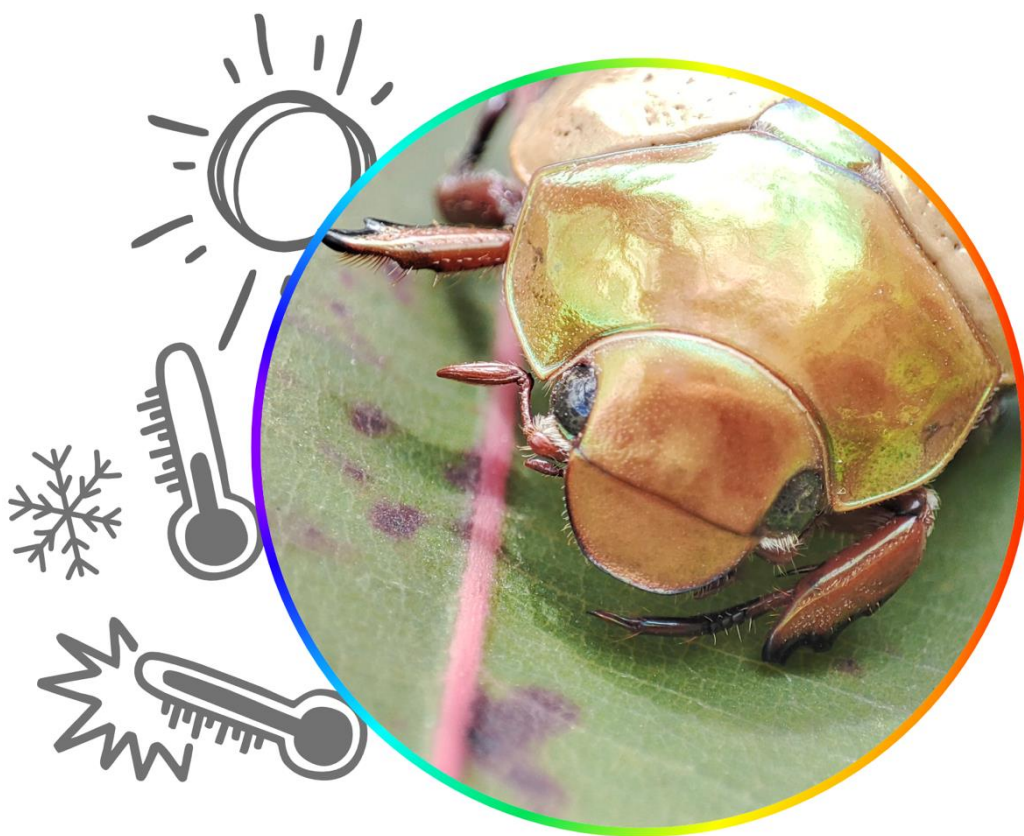
3.8 Acknowledgements

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Chapter 4 - Pretty cool beetles

Can manipulation of visible and near-infrared
sunlight prevent overheating?



*This work is presents as its published version: Laura Ospina-Rozo, Jegadesan Subbiah, Ainsley Seago, Devi Stuart-Fox, 2022. Pretty Cool Beetles: Can Manipulation of Visible and Near-Infrared Sunlight Prevent Overheating?, *Integrative Organismal Biology*, obac036, <https://doi.org/10.1093/iob/obac036>*

4.1 | Abstract

Passive thermoregulation is an important strategy to prevent overheating in thermally challenging environments. Can the diversity of optical properties found in Christmas beetles (Rutelinae) be an advantage to keep cool? We measured changes in temperature of the elytra of 26 species of Christmas beetles, exclusively due to direct radiation from a solar simulator in visible (VIS: 400-700 nm) and near infrared (NIR: 700-1700 nm) wavebands. Then, we evaluated if the optical properties of elytra could predict their steady state temperature and heating rates, while controlling for size. We found that higher absorptivity increases the heating rate and final steady state of the beetle elytra in a biologically significant range (3 to 5°C). There was substantial variation in the absorptivity of Christmas beetle elytra; and this variation was achieved by different combinations of reflectivity and transmissivity in both VIS and NIR. Size was an important factor predicting the change in temperature of the elytra after 5 min (steady state) but not maximum heating rate. Lastly, we show that the presence of the elytra covering the body of the beetle can reduce heating of the body itself. We propose that beetle elytra can act as a semi-insulating layer to enable passive thermoregulation through high reflectivity of elytra, resulting in low absorptivity of solar radiation. Alternatively, if beetle elytra absorb a high proportion of solar radiation, they may reduce heat transfer from the elytra to the body through behavioural or physiological mechanisms.

4.2 | Introduction

Solar radiation is an important selective pressure shaping the adaptations of organisms. Animals absorb, reflect, or avoid sunlight to maintain their temperatures in a functional range (Norris, 1967). In thermally challenging environments, even small variations in temperature ($\sim <3^{\circ}\text{C}$) produced by different optical properties can be biologically relevant to avoid overheating (Medina *et al.*, 2018; Munro *et al.*, 2019) or resist extreme cold (Zverev *et al.*, 2018). We use the term optical properties to encompass a wide diversity of interactions between natural materials and sunlight, i.e., reflectance, absorption and transmittance in different wavelengths of the solar spectrum. Such passive mechanisms of thermoregulation may be very important for ectotherms since their body temperature depends more directly on the environment (Careau *et al.*, 2015), especially in small animals with rapid rates of heat exchange such as insects (Casey, 1992; Amore *et al.*, 2017). However, the role of optical properties in thermoregulation is difficult to quantify since it may be masked by effects of conductive or convective heat, body size, shape and behaviour (Stuart-Fox *et al.*, 2017). In this study, we tested the potential of optical properties as a mechanism for passive thermoregulation in a set of closely related species of beetles with diverse optical properties that occur in challenging thermal environments.

In the absence of an insulating layer, both transmitted and absorbed light increase the temperature of the body due to direct penetration of radiation or conduction. Conversely, when an insulating layer is present, transmittance and reflectance both play important roles. For example, in polar bears (Preciado *et al.*, 2002) and sub-arctic mammals (Walsberg, 1991), the increased reflectance of the white fur (which aids in crypsis) is counteracted by maximized transmittance to increase the skin temperature. A similar case of balance between the two properties has been recently described for sunbirds' feathers (Rogalla *et al.*, 2021). To add another layer of complexity, optical properties may vary with wavelength. Transmittance is influenced by the light scattering properties of the material, both at the cuticle surface and within the integument. In the latter, longer wavelengths penetrate deeper because they are scattered less (Johnsen, 2012). Absorption of different wavelengths depends on scattering losses and the chemical composition of the underlying pigments (Johnsen, 2012), and in some species the reflectance of near infrared (NIR) wavelengths (700 - 2500 nm) seems to be largely independent of visible light (Stuart-Fox *et al.*, 2017). Both ultraviolet visible wavelengths (UV-VIS: 300-700 nm) and NIR wavelengths (NIR: 700-2500 nm) are important for radiative heat gain because each waveband accounts

for approximately half of the radiant energy in direct sunlight (Stuart-Fox *et al.*, 2017). Studying NIR is particularly relevant since these wavelengths are not likely to be constrained by selection for communication or camouflage (Stuart-Fox *et al.*, 2017). In consequence, different combinations of reflectance, absorption and transmittance in a wide spectrum should be considered in thermoregulation experiments.

There are very few studies that address the effect of transmittance on heat load in insects because as insulation decreases, the effect of reflectance should increase (Dawson *et al.*, 2014), and it is assumed that insects have comparatively little insulation. Beetles offer a curious case: although they lack a full-body insulation system like birds' feathers or cetaceans' blubber, their elytra (modified first pair of wings) fold on top of their secondary wings leaving an air gap between their outermost layer and the body, which may act as a buffer for heat transfer (Heinrich, 1993; Bolwig, 1957). Some beetles also possess abundant hair-like setae, but these are believed to be more closely tied to defensive mimicry than to thermoregulation (e.g. Glaphyridae: *Lichnanthe*, Carlson, 1980). In most beetles, the elytra are rarely opened outside of flight, and as a consequence they represent the largest area of the beetle exposed to sunlight (unlike butterfly wings in Tsai *et al.*, 2020) so their optical properties may play an important role in passive thermoregulation. Although several studies have explored passive thermoregulation in beetles (Carrascal *et al.*, 2017; Turner & Lombard, 1990; Zverev *et al.*, 2018; Willmer & Unwin, 1981; Schultz & Hadley, 1987), very few have isolated the role of near infrared properties (Wang *et al.*, 2021; Cuesta & Lobo, 2019) and most of them have been focused on reflectance (With some exceptions: Alves *et al.*, 2018; Cuesta & Lobo, 2019). Studying transmittance is important because it can play an additional role in passive thermoregulation for beetles: elytra with high reflectance can shield the body from radiation, but elytra with high absorption and low transmittance may trap the excess of solar energy and redirect it away from the body. Thus, some questions remain: how much variation occurs in both visible and NIR light reflectance/transmittance between species? What is the relative importance of transmittance and reflectance for passive thermoregulation by the elytra? How do the effects of optical properties on heating rates scale with body size?

Christmas beetles (Scarabeidae: Rutelinae) are a good model to address questions of passive thermoregulation because they live in environments in which overheating due to radiation is a significant problem. In Australia, the common name "Christmas beetle" is applied to several genera of Rutelinae scarabs (some genus of the subfamily

Melolonthinae, and in Tasmania, lucanid beetles in the genus *Lamprima*) and refers to the fact that the adults of these colourful insects emerge and are active only during the warm Christmas season, i.e. December through January (Hangay & Zborowski, 2010). During the Australian summer, the conditions can be very challenging: low humidity, high radiation and temperatures between 26°C and ~ 40°C (CSIRO Bureau of Meteorology, 2014). The adults are active during the day and are usually found on the top branches of eucalyptus trees in forests and woodlands (Britton, 2020). Similar to most beetles, they move slowly and fly only occasionally (Heinrich, 1993), which means they spend much of their life time exposed to solar radiation. In addition, the group has highly diverse optical effects including a wide range of NIR properties, iridescent, pearlescent and metallic appearances (Ospina-Rozo *et al.*, 2022b). The optical effects of Christmas beetle elytra are not notably sexually dimorphic and thus presumed not to be subject to sexual selection.

Here we studied the potential of the optical properties of the beetle elytra in facilitating passive thermoregulation, focusing on reduction of heat load. We conducted high precision experiments to measure small changes in temperature exclusively due to direct radiation from a solar simulator in 56 Christmas beetle specimens from 28 species in 9 genera (Table C-1). These taxa were selected because they represent the wide diversity in optical effects in this group. We tested whether the optical properties (absorption, reflectance and transmittance) of the beetle elytra predict heating (heating rate and total increase in temperature after a given interval of time) in different spectral bands (visible 400-700 nm, NIR 700-1700 nm and combined 400-1700 nm), while accounting for the effect of size. In addition, we explored correlations between optical properties in different spectral bands. Finally, we quantified the effect of the elytra on the heating rate of the body for one species, *Anoplognathus chloropyrus*. According to the fundamental laws of thermodynamics optical properties are inevitably correlated with heating in a controlled set up (minimal convection or conduction), but from the biological point of view, it is critical to examine if the interspecific variation produces ecologically relevant differences in heating. It is also important to evaluate if the contributions of visible and near-infrared reflectivity vary between species given the high diversity of optical properties of Christmas beetles. We discuss how the interactions between the three optical properties of absorption, reflectance and transmittance influence passive thermoregulation by beetle elytra.

4.3 | Methods

4.3.1 | Museum specimens

We studied 56 Christmas beetles representing 28 species obtained from the Australian National Insect Collection (ANIC) (Table C-1). The elytra of the selected taxa have relatively smooth, waxy surfaces without scales or hair. We took photographs of one specimen of each species with a Nikon D7200 DSLR camera and a scale to measure the length of the beetle (in cm). This measurement is a good proxy for elytral area and body size in this group of beetles since their shape is very conserved. Our study consisted of two sets of experiments. In the first, we measured the heating of a single elytron removed from each museum specimen ($n = 56$ individuals, 28 species). In the second experiment, we examined how elytral heating rate corresponds to body temperature in one species, *Anoplognathus chloropyrus* ($n = 11$ individuals) for which we compared the heating of the body (inserted thermocouple, see below) with and without the elytra. Individuals of *A. chloropyrus* were collected in Orange - New South Wales (33.3234°S, 149.0828 °E. Collection permit number, FO25000127) in December 2019, and kept in captivity, with heating rate measured soon after their natural death. We used recently dead beetles to avoid error in our measurements due to movement or misplacement of thermocouples, ensure that our experiments excluded additional physiological mechanisms for heat transfer apart from direct radiation, and avoid the infliction of non-trivial discomfort to living beetles.

4.3.2 | Optical properties

To measure transmittance/direct transmission (Johnsen, 2012) we used a standard set up in which the spectrometers and light source are carefully aligned to send a parallel beam of light through the sample and collect, on the opposite side, the portion of the beam that was not absorbed or scattered (**Figure C-1**). We detached the elytra from the body of the beetle and placed one elytron between the light source and spectrometers. We used two light sources to include the UV-Visible range (PX-2 pulsed Xenon light) and the visible-NIR range (HL-2000 tungsten halogen light), combined via bifurcated optical fibres to illuminate the sample from the dorsal side of the elytra. We used the same two spectrometers connected to a bifurcated optical fibre to capture the light on the other side of the sample. Measurements were recorded in the same software and calibrated against the 100% reference (parallel beam of light without the sample).

Next, we calculated the total percentage of light transmitted or reflected, accounting for the spectral power distribution of the light source. This percentage is termed reflectivity or transmissivity respectively (definitions and formulae in Table 4-1). We calculated reflectivity and transmissivity for the different spectral bands: visible (VIS, 400-700 nm), near infrared (NIR, 700-1700 nm) and total (Total, 400-1700 nm). The visible spectral band includes 43.1% solar energy, while the near infrared band up until 1700 nm accounts for 46.9%. Thus, the spectral range considered in our experiment accounts for ~90% of solar energy and the total light incident over the samples is similar to that expected under the sun for each of the studied spectral bands (**Figure C-2**). In addition, the sum of the transmittivity and reflectivity is equivalent to all the light that is not absorbed by the material (Table 4-1); therefore, we calculated the percentage of absorptivity for the different spectral bands.

Table 4-1. Optical properties

This table contains the definitions (Johnsen, 2012) of the optical properties we studied and the equations to calculate the optical properties accounting for the spectral power distribution of the light source and filters used (Smith *et al.*, 2016b).

Optical Property	Symbol [Units]	Definition	How it was obtained
Irradiance	I [W/m ²]	The number of photons emitted by a source and received by a surface per unit of area	From the manufacturer of the solar simulator
Reflectance	E [%]	Radiance of an illuminated object normalized by the radiance of a standard illuminated by the same light	Measured with an integrating sphere from the elytron
Reflectivity	R [%]	Fraction of the incident radiation that is reflected by an object	$R = \frac{\int_i^n I(\lambda) F(\lambda) E(\lambda) d\lambda}{\int_i^n I(\lambda) F(\lambda) d\lambda}$
Transmittance	Sample = S [%] Filters = F [%]	Amount of light passing through an object normalized by the total amount of light shone on one of the surfaces of that object	Measured with a spectrometer from the elytron and the filters of the set up
Transmissivity	T [%]	Fraction of the incident radiation that is transmitted by an object	$T = \frac{\int_i^n I(\lambda) F(\lambda) S(\lambda) d\lambda}{\int_i^n I(\lambda) F(\lambda) d\lambda}$
Absorptivity	A [%]	Fraction of the incident radiation that is absorbed by an object (determined by its material and geometry)	$A = 100 - (T + R)$

4.3.3 | Heating experiments

As the light source, we used a solar simulator (model number: 91192, Oriel 104 Class A, with AM 1.5 filter; Newport Corp., Irvine, CA, USA) with energy density of 500 W/m² (0.5 Sun) that resembles the solar power distribution (**Figure C-2**). We obtained the irradiance spectrum of the solar simulator from the manufacturer for the wavelength range 300 - 1700 nm (**Figure C-2**).

To isolate the effect of the radiation emitted from the solar simulator, we placed the samples in a closed glass thermal chamber directly under the solar simulator, to ensure the sample was illuminated from above while controlling heat exchange by conduction or convection (**Figure 4-1**). We installed a glass water jacket to allow permanent flow of cold water around the chamber. By controlling the temperature of the water bath (measured with a thermocouple) it was possible to keep the temperature of the air inside the chamber constant (measured with a second thermocouple). Inside, samples were placed in the centre of a transparent acrylic platform and connected to a thermocouple (ca. 5 mm TP-K01, K type, Center Technology Corp., Taiwan) to record the temperature change throughout the experiment. The thermocouples were connected to a thermometer (Center 521, Center Technology Corp., Taiwan) recording their measurements every 20 seconds. The glass chamber had one glass portal on top to control the illumination conditions inside the chamber. For full spectrum illumination conditions, the silicon glass portal was uncovered allowing all wavelengths emitted by the solar simulator to be transmitted and illuminate the sample (abbreviated TOTAL). For NIR (700 to 1700) and VIS (400 to 700 nm) illumination conditions, custom optical filter combinations (Edmund Optics) were placed on top of the portal (details in **Figure C-2B-C**) to only allow transmission of the relevant wavelengths. For periods that required the absence of light (cooling), a lid was placed on top of the portal.

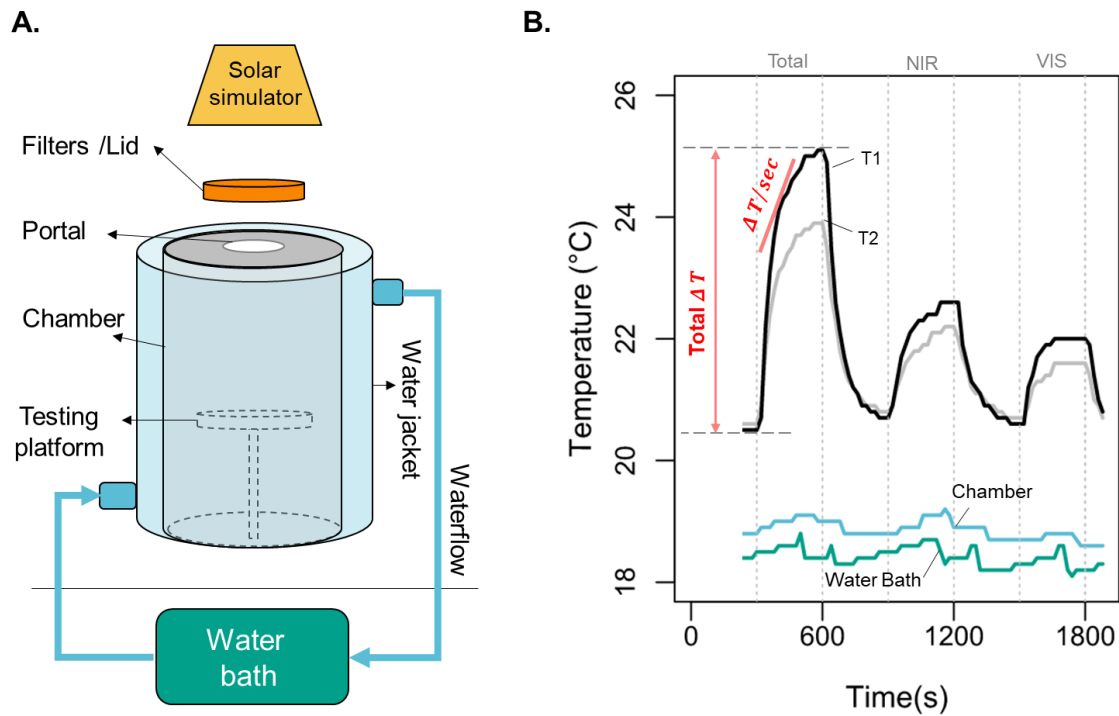


Figure 4-1. Heating rates experimental set up

A. We used a solar simulator as the illumination source, filters to test different spectral bands, and a thermal chamber to control the effect of convection or conduction. **B.** Example of one cycle of measurements for two samples (T1 and T2). The temperature of the water and the chamber remained constant, while the temperature of the samples increased under different illumination conditions in intervals of 5 minutes. We used two measurements of heating (red): total change in temperature (ΔT_5) and heating rates ($\Delta T/sec$). The latter was calculated for every pair of points along the heating curve, but only the maximum slope (maxHR) was used in our analyses.

▪ Single elytron

In order to study the effect of radiation on a single elytron of different beetle species ($n = 56$), we placed two elytra in the centre of the platform inside the chamber and installed one thermocouple touching the ventral surface of each of them (i.e., we conducted the experiment for two elytra at a time). Although it was expected that the area under the glass portal was evenly illuminated, we recorded the side on which the elytra was placed and included it in our analyses to account for any possible effect of this variable. Each trial consisted in alternating 5 minutes heating and cooling periods in a sequence of: initial cooling, TOTAL, cooling, NIR, cooling, VIS, cooling (Figure 4-1B). The initial cooling period was prolonged until the samples reached a stable temperature around 20 degrees. Cooling periods between the different illumination conditions were required to ensure the sample always returned to a similar temperature before starting a new illumination condition.

■ Beetle body experiments

The biological significance of the heating of the elytra depends on the heat transfer between the elytra and the body of the beetle. To confirm whether the elytra play a role in insulating the body, we checked if the presence of the elytra affects body temperature. We placed recently dead individuals of *Anoplognathus chloropyrus* (n = 11) in the centre of the chamber and introduced a thermocouple inside its body through the posterior end of the abdomen. We chose this species because it corresponds closely to the average reflectance for our sample in both visible and NIR reflectance: it has a light brown appearance corresponding to a broad band relatively low reflectance and transmittance in the visible spectrum and medium NIR reflectance (Figure 4-3B). For these experiments each trial consisted of the same sequence mentioned above allowing the same 5 minutes of radiation and 10 minutes of cooling periods because the body has a larger mass and takes longer to dissipate heat. For each individual, we conducted one trial with the elytra closed and one with the elytra open immediately after (Figure 4-4A) to compare the heating of the same mass with and without the elytra.

4.3.4 | Heating estimates

We used two different parameters to quantify heating: the total change in temperature after 5 minutes of illumination (ΔT_5 , °C/ 5 min) and the maximum heating rate (maxHR = $\Delta T/sec$, °C/sec). The latter was calculated as the maximum slope between two adjacent points of the heating curve divided by 20 seconds (thermometer data collection interval) (Figure 4-1B). These two parameters are expected to be affected differently by the size of the object: large objects are expected to have greater steady state temperatures but slower heating rates than small objects. It is reasonable to assume that ΔT_5 is indicative of the steady state temperature since most of our samples started to plateau after 5 minutes under any of the three illumination conditions.

4.3.5 | Statistical analysis

All statistical analyses were conducted in R 4.1.2 (R Core Team, 2021). We conducted a repeatability test on repetitions of the ΔT_5 and maxHR measurement for the elytra of *A. aureus* (n = 7), *P. olivaceous* (n = 7), *A. prasinus* (n = 9), *C. rayneri* (n = 7), *R. manicatus* (n = 6) and *A. viriditarsis* (n = 8), for each of the spectral bands (TOTAL,

NIR, VIS). We used a bootstrapping tool for gaussian data and calculated the percentage of variance explained by the groups and the p-value based on a likelihood ratio test (rpt, R package rptR; (Stoffel *et al.*, 2017).

- **Single elytron experiments**

We used linear models to test if elytra absorptivity (n=56 beetle elytra) predicted ΔT_5 and maxHR under different illumination conditions (TOTAL, NIR and VIS). We included the size of the elytra as a fixed effect, since it was expected to influence heating rates due to differences in mass and area. We did not include interaction terms in our models because there was no *a priori* reason to expect such interaction: in our set up, the thermodynamical law of conservation of energy can be applied since any increase in temperature (molecular kinetic energy) is a consequence of the absorption of radiative energy (Shankar, 2014). Increased absorption of light can only be produced by increased absorptivity per unit of area (as measured here) or increased total area exposed (here size). Thus, as long as the illumination is even across the surface (not a laser or a focused beam), the heating of the sample depends on the additive effects of absorptivity and size, while synergistic or antagonistic effects (interactions) are not expected. Importantly, the absorptivity of a material depends on its refractive index, the optical properties of its pigments and the geometry of its structures. The latter can be altered by the size of the object, but this is still an additive effect since ultimately it will affect the total amount of light absorbed. After fitting the linear models, we checked the distribution of the residuals to confirm the assumption of normality with mean = 0 and constant variance. We used Pearson's correlation to explore correlations between transmissivity and reflectivity, between different spectral bands, and between elytra optical properties and size. We did not use phylogenetic correction because our experiment is designed to explore the biophysical relationship between heating rates and optical properties of the materials, which is independent of any evolutionary associations. That is, heating rate is a physical consequence of reflectivity, rather than a trait that can evolve independently from reflectivity (or evolve together with reflectivity in response to similar selective pressures); therefore, it is not appropriate to test for correlated evolution of reflectivity and heating rate using phylogenetic comparative methods. Additionally, the optical properties of Christmas beetles' elytra vary greatly among congeneric species and show no evidence of clustering by genus. (Ospina-Rozo *et al.*, 2022b).

- Beetle body experiments

We tested if there is a greater ΔT_5 in the beetle body when the elytra are not covering the body by using a non-parametric paired analysis. For each individual, we calculated the difference in ΔT_5 when its elytra were open and when they were closed ($D_{\Delta T}$) (equation in Figure 4-4A). Then, we used bootstrapping to estimate a 95% confidence interval around the mean of the difference ($D_{\Delta T}$) in a group of 11 recently dead beetles. A negative interval not including 0 indicates that the change in temperature of the beetle body is greater when the elytra are not covering it. We repeated this procedure for each of the three spectral bands.

4.4 | Results

4.4.1 | Repeatability of the heating measurements

Repeatability was high for the measurements of ΔT_5 in the three illumination conditions: TOTAL $R = 0.91$, NIR $R = 0.79$ and VIS $R = 0.78$ (Table C-2). This result, along with the small standard error in our estimates in the linear models (Table 4-2) showed that our set up is reliable to detect very small changes in temperature due to radiative heat in the range 0.7 to 5.3 °C/5 min. Repeatability of maxHR was considerably lower: TOTAL $R = 0.42$, NIR $R = 0.64$ and VIS $R = 0.22$ (Table C-2). This result is expected since the calculation of maxHR was obtained as the derivative of the heating curves at the very fine scale of 0.01 and 0.105 °C/s. However, our analysis also showed that despite the low values of repeatability for this calculated value, the differences between species are still detectable in the data set (p value for TOTAL and NIR <0.01 and for VIS a marginal 0.059; raw results for both heating measurements in Figure C-3). Due to low repeatability of the maximum heating rate, our inferences are based more strongly on the final temperature.

Table 4-2. Absorptivity as a predictor of heating

Results of 6 linear models testing how heating (ΔT_5 and maxHR) is predicted by absorptivity and size (elytra length) in each spectral range.

Response variable	Spectral Range	Overall R ² (%)	Parameter	Partial R ² (%)	Estimate	Std Error	p-value
ΔT_5 (°C/5 min)	Total	53.65	Intercept	NA	2.024	0.334	< 0.001
			Absorptivity	47.08	0.029	0.004	< 0.001
			Size	4.67	0.274	0.124	< 0.05
	NIR	32.17	Intercept	NA	1.13	0.192	< 0.001
			Absorptivity	30.57	0.010	0.002	< 0.001
			Size	0.97	0.069	0.083	0.412
	VIS	43.43	Intercept	NA	0.201	0.235	0.396
			Absorptivity	14.08	0.008	0.002	< 0.05
			Size	20.76	0.222	0.053	< 0.001
maxHR (max. Δ °C/ sec)	Total	29.91	Intercept	NA	0.0322	0.0125	< 0.05
			Absorptivity	22.53	0.0006	0.0002	< 0.001
			Size	1.1	0.0036	0.0046	0.432
	NIR	30.33	Intercept	NA	0.0263	0.0043	< 0.001
			Absorptivity	22.21	0.0002	0.0001	< 0.001
			Size	4.28	-0.0035	0.0019	0.067
	VIS	24.27	Intercept	NA	0.0026	0.0097	0.788
			Absorptivity	11.62	0.0003	0.0001	< 0.05
			Size	0.34	0.0007	0.0022	0.763

Note: The three spectral bands were TOTAL (400 - 1700 nm), NIR (700 - 1700 nm) and VIS (400 - 700 nm). Units of the estimates and standard errors are specified in the response variable column. The reported estimates already account for the effect of the side of the platform in which the samples were placed. The difference between the overall R² and the sum of the partial R² corresponds to the variance (%) explained by the side. Its effect was significant (p < 0.05) only for ΔT_5 VIS, and maxHR TOT and VIS.

4.4.2 | Effect of optical properties on the heating of single elytra

We found that higher absorptivity significantly increased both ΔT_5 and maxHR in all three illumination conditions (Figure 4-2; Table 4-2) after controlling for size. Absorptivity explained 14% - 47% of the variation in ΔT_5 , while reflectivity by itself

explained only 5% to 21%. Absorptivity explained 11% - 23% of the variation in maxHR, while reflectivity by itself explains 8% - 21% (partial R^2 in Table C-3). Transmissivity by itself was not a significant predictor of the heating measurements (Table C-4). Our results show that combining transmissivity and reflectivity to estimate absorptivity improved the power of our models to predict ΔT_5 and maxHR.

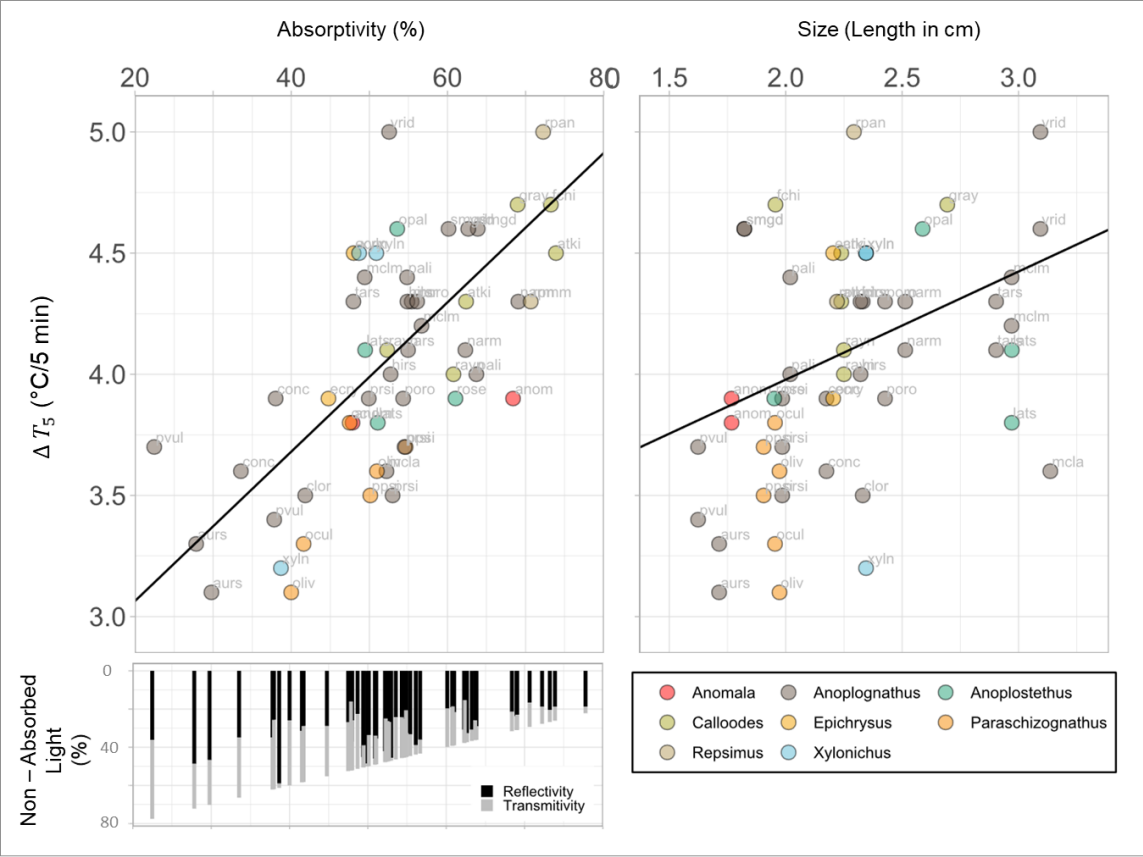


Figure 4-2. Example of the effect of absorptivity and the size on heating of single elytra

Higher percentages of absorptivity and larger elytra predict a greater increase in temperature due to radiative heat gain in the Total spectral band from 400 to 1700 nm (top panels). Variation in the absorptivity of Christmas beetle elytra is produced by different combinations of transmissivity and reflectivity (bottom left). We sampled 28 species from 8 genera of Christmas beetles (bottom right).

4.4.3 | Correlations between the optical properties of the elytra

A negative correlation between reflectivity and transmissivity was expected since the light that is reflected cannot be transmitted by the elytra. Interestingly, we only found a significant correlation between reflectivity and transmissivity in TOTAL (-32 %, $p < 0.05$) and NIR (-47%, $p < 0.01$) spectral bands, but not in VIS ($p = 0.84$; Table 4-3). We observed considerable variation in the combinations of transmissivity and reflectivity in the two spectral bands studied (NIR and VIS; Figure 4-3). In addition, we observed that the percentage of light absorbed by the beetle elytra was much higher in VIS than

in NIR (Figure 4-3). Reflectivity in VIS ranged between 5 to 30 %, while in NIR it ranged from 25 to 90%, and on average transmissivity was also higher in NIR than in VIS. We evaluated the extent to which the optical properties in the two different spectral bands (VIS and NIR) may be correlated for this group of beetles (Table 4-3). A correlation between NIR and VIS light manipulation is expected, since light manipulation depends on structures or compounds tuned to specific wave bands; however, the strength of this correlation may vary in different groups of animals. We found a strong correlation between NIR and VIS transmissivity (Pearson's coefficient $R = 0.75$) and absorptivity (Pearson's coefficient $R = 0.78$). However, the correlation between NIR and VIS reflectivity was only $R = 0.58$. This result indicates that for some species high reflectivity in NIR is accompanied by low reflectivity in VIS or vice versa.

Table 4-3 Correlations between parameters

Pearson's correlation coefficient, confidence intervals and p-values are given.

Correlations involving:	Parameters	Spectral Range	Pearson's R	95% confidence interval	p-value
Optical properties	Transmissivity x Reflectivity	Total	- 0.325	-0.542 , -0.068	0.014
		NIR	- 0.477	-0.654 , -0.239	< 0.001
		VIS	0.027	-0.289 , +0.237	0.842
Spectral Bands NIR - VIS	NIR - VIS Transmissivity	-	0.750	+0.607 , +0.846	< 0.001
	NIR - VIS Reflectivity	-	0.585	+0.381 , +0.735	< 0.001
	NIR - VIS Absorptivity	-	0.782	+0.653 , +0.866	< 0.001
Size and optical properties	Size x Reflectivity	Total	0.124	-0.143 , +0.375	0.361
		NIR	0.130	-0.137 , +0.379	0.339
		VIS	0.062	-0.204 , +0.319	0.651
	Size x Transmissivity	Total	-0.343	-0.556 , -0.088	0.009
		NIR	-0.364	-0.572 , -0.112	0.006
		VIS	-0.204	-0.443 , +0.062	0.131
	Size x Absorptivity	Total	0.197	-0.068 , +0.438	0.144
		NIR	0.247	-0.017 , +0.479	0.066
		VIS	0.076	-0.191 , +0.332	0.578

4.4.4 | Effect of size on the heating rate of single elytra

Larger elytra had a greater ΔT_5 in TOTAL (example in **Figure 4-2**) and VIS but not under NIR illumination (**Table 4-2**). We did not find evidence of an effect of size on the maxHR in any of the three illumination conditions (**Table 4-2**). These results show that the variation in size in this group is only a significant predictor of variation in the final temperature after 5 minutes, but not in heating rates. Size was not correlated with reflectivity nor absorptivity, but it was negatively correlated with transmissivity in TOTAL (-34 %) and NIR (-36 %) spectral bands (**Table 4-3**). Thus, only the transmissivity in NIR scaled with the size of elytra, while the other optical properties were independent of elytra size, for the modest degree of size variation in our dataset.

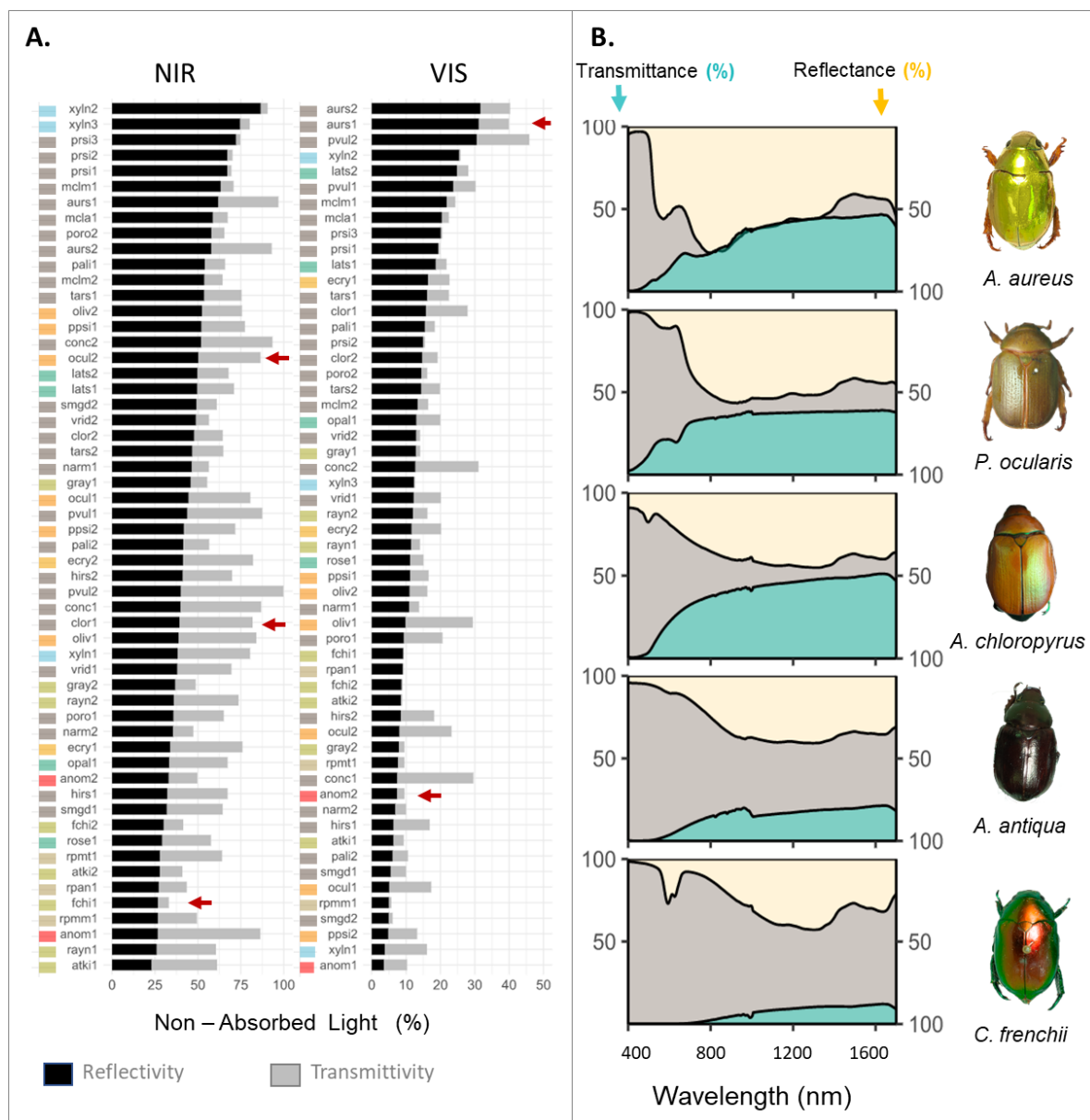


Figure 4-3. Variation in optical properties of Christmas beetle elytra in NIR and VIS light

A) In general, elytra absorb a higher percentage of VIS wavelengths than NIR wavelengths (note difference in x axis). Colours correspond to the genus (consistent with Figure 4-2, bottom right). B). Raw spectra of some of the studied species (also marked in panel A with red arrows). Reflectance is always at the top with an inverted axis. Grey represents the light absorbed by the elytra.

4.4.5 | Effect of the elytra in the heating rate of the full body

The body of *A. chloropyrus* had higher ΔT_5 when it was not covered by the elytra, i.e. negative difference in the paired experiment (**Figure 4-4A**), in TOTAL (95% C.I. -0.482, -0.127) and NIR (95% C.I. -0.318, -0.136) illumination, but not in VIS (95% C. I. -0.0182, 0.127) since the 95% C. I. overlapped with 0 (**Figure 4-4B**).

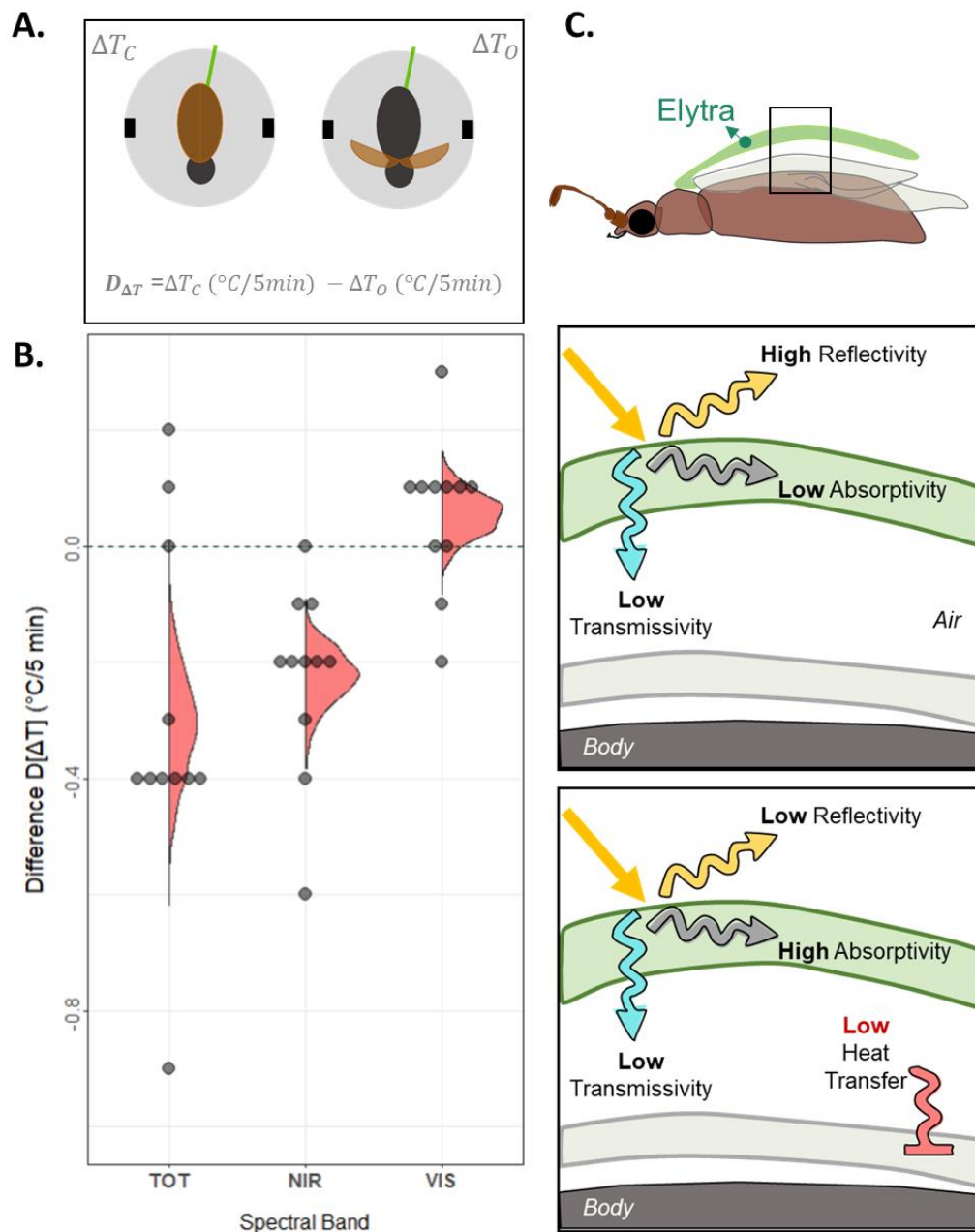


Figure 4-4. Effect of the elytra on heating of the body of *Anoplognathus chloropyrus*

A. Graphical representation of our test statistic for the non-parametric paired test. B. $D_{\Delta T}$ for each spectral band. A negative value indicates that the increase in temperature of the beetle body is greater when the elytra are not covering it. The half violin plots represent the results of the bootstrap loop. $D_{\Delta T}$ is significantly different to 0 only under full and NIR illumination. C. Two potential strategies to avoid overheating. Beetles could have elytra with high reflectivity or low transmissivity, both resulting in low heat transfer from the elytra to the body.

4.5 | Discussion

Are pretty beetles also cool beetles? The astonishing diversity in appearances of Christmas beetles is determined by the way their cuticle interacts with light. We evaluated how different optical properties of beetle elytra affect heating due to radiation in different spectral bands: visible (400-700 nm) and NIR (700-1400 nm). We found that absorptivity (the combination of reflectivity and transmissivity) is a better predictor of the temperature and heating rate of Christmas beetle elytra than reflectivity. Lower absorptivity corresponds to lower heating rate (maxHR) and cooler final steady state (ΔT_5) of the elytra. Christmas beetles vary greatly in their absorptivity by combining different degrees of reflectivity and transmissivity in both VIS and NIR. Their optical properties are only weakly correlated in NIR and not correlated in VIS, while high reflectivity in NIR is not restricted to species with high reflectivity in VIS (58% correlation). Moreover, relatively small variations in size amongst species can also affect heating. Consequently, different species of Christmas beetles can have elytra with different potential for passive thermoregulation and achieve this by different combinations of optical properties. Since the presence of the elytra covering the body of the beetle can reduce the heating of the body itself, we propose that the interactions of the beetle elytra with light could help some species to keep cool.

Our experiment was able to detect very small changes in temperature due to radiation, but are these changes biologically significant? The final steady state of the elytra ΔT_5 ranged from 3°C to 5°C, while having the elytra folded on top of the body reduced the ΔT_5 of the body of *A. chloropyrus* by 0.2 degrees. These changes may seem smaller than the difference of 5°C between treatments found by (Carrascal *et al.*, 2017 - Comparison: final temperature after 8 minutes of natural light illumination ventral vs dorsal orientation). However, our glass chamber was designed to minimize heating by conduction or convection and our initial temperature was kept at 20°C for all experiments. If these two variables are not controlled, air temperature can rapidly

increase due to the heat generated by the light source. Conduction can then contribute to the heating of the sample, inflating the estimated effect of reflectivity. Conversely, our results reflect only the differences caused by radiative heat, which can be a decisive factor for passive thermoregulation in hot environments (Shi *et al.*, 2015).

The differences of 3°C to 5°C in ΔT_5 we registered between beetle elytra are within the range of other biologically significant values. For example, a ~5°C increase in temperature can make the difference between non-active to active status in jewel beetles (Bonsignore & Bellamy, 2007) and a decrease in temperature of ~1.5 and 6°C can make the difference between thoracic temperature during and post flying (as an indication of a cooling down process) in burying beetles (Merrick & Smith, 2004). In a similar way, relatively small temperature differences could make the difference between remaining at a peak of optimal performance temperature and reaching a critical upper thermal limit. Although the difference in body temperature with and without elytra that we registered (0.2°C) is small, *A. chloropyrus* has moderate reflectivity, and the difference in ΔT_5 for species with higher reflectivity is expected to be higher. Thus, the optical properties of the elytra could influence the beetles' fitness by affecting the amount of time spent feeding or mating rather than sheltering to avoid overheating (Clusella Trullas *et al.*, 2007; Umbers *et al.*, 2013). Further experimental studies are required to quantify the contribution of these optical properties relative to other factors such as metabolic production of heat, body mass, shape and behaviour, to ultimately estimate their effect on the fitness of individuals.

An outstanding question about the relevance of light manipulation for thermoregulation is: what is the correlation between optical properties and to what extent do they vary across different spectral bands in a given taxonomic group (Stuart-Fox *et al.*, 2017)? Previous studies comparing visible and infrared light reflectance and transmittance have had limited capacity to address this question because they focus on a relatively small number of species (eg. Amore *et al.*, 2017; Cuesta & Lobo, 2019 - 7 and 2 species respectively). In our study we evaluated 28 optically diverse, closely related species. We found that, unlike other groups, Christmas beetles can achieve a wide range of combinations of optical properties in different spectral bands. For example, reflectivity and transmissivity are expected to be negatively correlated, but they showed only a weak correlation in near infrared and no correlation in visible wavelengths. In a similar way, the optical properties in visible and near infrared are expected to be correlated (for example, a highly reflective red patch is expected to also have some reflection in the near infrared). Our results supported this prediction,

but the correlation between NIR and VIS was notably higher for transmissivity (~75%) and absorptivity (78%) than reflectivity (58%). This may be explained by their underlying mechanisms. Transmittance and absorption may be primarily determined by cuticle thickness and presence or absence of underlying pigments, whereas reflectance is likely to involve modular and hierarchically ordered nanostructures optimized to interact with different spectral ranges. As a result, reflectance in visible and infrared spectral bands can vary more independently in response to selection for different functions. This has also been observed in other species such as birds (Stuart-Fox *et al.*, 2018), butterflies (Tsai *et al.*, 2020) and chameleons (Teyssier *et al.*, 2015).

Our results suggest that the metallic appearances associated with broadband reflectance (such as gold, silver, and brassy) of some beetles have higher potential for passive thermoregulation than narrow-band structural colours. Metallic colours, produced by multilayer reflectors in the beetle elytra (Seago *et al.*, 2009; Thomas *et al.*, 2007), have traditionally been considered to be unrelated to thermoregulation (Heinrich, 1993). However, in our sample, strongly metallic species such as *A. aureus* and *A. parvulus*, have broadband and high overall reflectivity with corresponding low heating, while species with narrow band deep green colours (e.g. *A. smaragdinus*, *C. atkinsoni* and *C. rayneri*) have low reflectivity and comparatively high heating due to radiation. Some species such as *A. viriditarsis*, *A. chloropyrus* and *A. porosus* have a sheen of metallic colour, but the heating of their elytra is more strongly determined by the total reflectance arising from the underlying brown (melanin) layer than by the top layers producing the metallic sheen. Although in some studies it has been mentioned that “metallic” colours can reach very limited reflectance (Alves *et al.*, 2018), this may be because the term was used to refer to narrow-band iridescent colours. Instead, the nanostructures that produce metallic gold appearances can result in high reflectivity across a broad range of wavelengths (Feller *et al.*, 2017; Parker *et al.*, 1998). Although the biological significance of these highly metallic appearances is still unclear (Thomas *et al.*, 2007; Franklin *et al.*, 2021), it may be premature to exclude a thermal function. Indeed, if high-reflectance optical mechanisms are effective in preventing the insect from overheating, this could be a factor explaining the apparent concentration of bright metallic-coloured insects in the warmer latitudes of the globe.

We demonstrated that it is important to consider NIR wavelengths in studies of radiative heat gain. The optical properties in these wavelengths predict the heating rates and final temperature of both the elytra and the beetle body. Most previous

studies of passive thermoregulation in insects have not isolated the effect of visible light from the near infrared wavelengths (Umbers *et al.*, 2013). The studies that do consider these different wavebands provide mixed evidence: for tiger beetles, NIR reflectivity seems to be present only in white patches and has a limited role in thermoregulation (Schultz & Hadley, 1987), while jewel beetles have high variability in NIR reflectance, which in turn, has a strong effect on the heating rates of their elytra (Wang *et al.*, 2021). Our sample consisted of species with high diversity in NIR reflectivity ranging between 23% - 86%, and NIR transmissivity ranging from 2% - 60%. We found that the lowest heating rates and final temperature of the elytra were observed for species with the lowest NIR absorptivity, achieved either by high NIR reflectivity (*X. eucalypti*, *A. prasinus*, *A. aureus* and *P. olivaceous*) or high NIR transmissivity (*A. concolor* and *P. ocellaris*). Therefore, we conclude that manipulating NIR wavelengths can be an effective mechanism to aid in passive thermoregulation for some species of Christmas beetles. The importance of the NIR wavelengths will depend on the biology of the species and the variability in thermal environments and optical properties. Future studies could explore to what extent NIR reflectance and transmittance vary in the different families of beetles and whether this corresponds to specific thermal environments.

In addition to the optical properties, size is also a relevant factor for thermoregulation. Generally, larger organisms are predicted to have a higher steady state temperature, but slower heating rates and vice versa (Porter & Gates, 1969; Dzialowski, 2005). In our experiment, size predicted ΔT_5 but not heating rates. In jewel beetles (length~ 1.2 to 4 cm) , size (effective area) predicted heating rates of isolated elytra under full spectrum illumination (Wang *et al.*, 2021). Despite testing a larger sample size, we found no effect of size on heating rates possibly because Christmas beetles are more homogeneous in size (~ 1.6 to 3 cm length) and shape. On the other hand, size significantly determined ΔT_5 under VIS, but not under NIR radiation. This may be because absorptivity was higher in VIS than NIR, and a larger area of elytra with high absorption is associated with a higher increase in temperature (Stewart & Dixon, 1989), making the effect of size easier to detect. Finally, the negative correlation between size and NIR transmissivity may be because longer wavelengths penetrate deeper in the structures undergoing more scattering/absorption in a thicker, more complex cuticle likely associated with larger elytra (Johnsen, 2012; Cuesta & Lobo, 2019).

Traditionally, only reflectance is considered in thermoregulatory studies for insects assuming a lack of insulation. Alves *et al.* (2018) suggested that higher transmittance

from the ventral side to the exterior of the elytra could facilitate heat dissipation. Although we found differences in transmittance of light incident on the dorsal and ventral sides of the elytra (**Figure C-4**), under natural circumstances, light in the solar spectrum would not be transmitted from the ventral side when the elytra are closed. Additionally, optical properties in the solar spectrum are not relevant to heat dissipation from the body to the environment, which is a function of emissivity in the mid- to long wavelength range, i.e. 4000 to 50000 nm (Nussear *et al.*, 2000). A recent study on heat transfer through the iridescent feathers of sunbirds showed that the melanin granules (whose ordered arrangement produces the iridescence) enhance absorption and reduce transmittance of light towards the skin potentially preventing heating up if combined with mechanisms to reduce heat transfer to the skin (Rogalla *et al.*, 2021). Our results showed that combining reflectance with transmittance (i.e., absorption) helps to better predict the heating in this group of beetles in contrast to only considering reflectance. Elytra with high reflectance can reduce heating, but elytra with high absorption could still achieve a similar result if combined with a mechanism to reduce heat transfer towards the body, and this is only possible because of their low transmittance. Such mechanisms can include morphological strategies such as increasing the size of the air layer underneath the elytra (Heinrich, 1993), as well as physiological or behavioural strategies to allow rotation of the air such as controlled breathing (Duncan, 2021) and periodically opening the elytra (“ventilations” in Bolwig, 1957). Thus, two strategies could be effective for passive thermoregulation by the elytra to avoid overheating: Christmas beetles may either use their elytra as a shield against radiative heat gain or employ physiological or behavioural mechanisms to dissipate the radiative heat absorbed by the elytra. Whether both strategies are used by species in thermally challenging environments requires observation of beetles in their natural environment.

4.6 | Data availability

In support of the Open Science Framework, find here a link and QR code to interactive web pages of my statistical analysis. This resource also provides access to the original data and permanent version tracking.

[Pretty cool beetles code](#)



4.7 | CRediT Author Statement

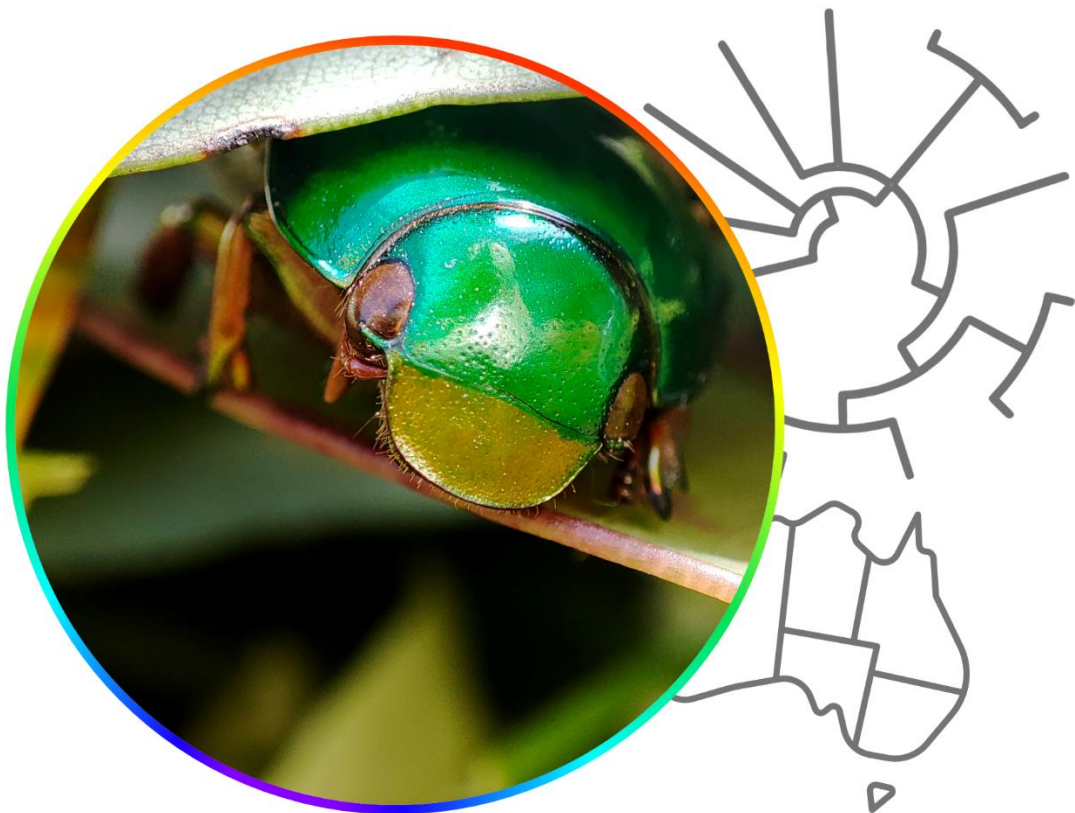
Laura Ospina-Rozo: conceptualisation, investigation, formal analysis, visualisation and original draft. **Jegadesan Subbiah:** Methodology, validation, Resources, Review & Editing. **Ainsley Seago:** Review & Editing. **Devi Stuart-fox:** conceptualisation, writing-review and editing, supervision.

4.8 | Acknowledgements

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Chapter 5 - Photonic Rebels

Christmas beetles' reflectivity, absorptivity and polarisation do not follow one universal ecogeographical rule



5.1 | Abstract

The brilliant and colourful appearances of some beetles have been abundantly studied to understand their underlying structural basis. However, little is known about the ecological variables driving the evolution of these optical effects. We aimed to identify environmental correlates for reflectivity, absorptivity and circular polarisation produced by a combination of pigments and structures in optically diverse Christmas beetles (Scarabeidae - Rutelinae). We quantified the optical properties of 261 specimens representing 46 species using spectrophotometry and calibrated photographs. Then, we examined the correlations between these properties and their associations with environmental variables such as temperature, humidity and vegetation cover, correcting for size and phylogenetic relatedness. Our results showed that body size and reflectivity in the visible spectrum are associated with humidity rather than temperature, with larger beetles having higher reflectivity and occurring in drier environments. Neither NIR reflectivity nor NIR absorptivity correlated with ecological variables, suggesting that selection for thermal benefits is not a strong driver of optical properties in this group of beetles. The degree of polarisation was negatively associated with NIR reflectivity but not with ecological variables or visible reflectivity, which may be more strongly affected by pigments such as melanin than polarisation-associated nanostructures. Our study highlights the value of a macroecological approach for testing alternative hypotheses to explain the diversity of optical effects in beetles and to understand the link between structure and function.

5.2 | Introduction

Beetles are the most successful animal on earth: almost one quarter of all living eukaryotes are beetles (Stork, 2018). A key to their success is the modification of their second pair of wings (elytra) into a shield that protects the forewings, allowing them to colonise every non-marine environment without compromising their ability to fly (Lawrence & Newton, 1982). The diversity of colours and optical effects produced by beetle elytra has captured the attention of biologists for centuries (Raupach, 2018; Rivers, 2003; Schroeder *et al.*, 2018; Srinivasarao, 1999). These effects include striking iridescence, gold/silver appearance, mirror-like effects and pearly colours (Berthier, 2007; Seago *et al.*, 2009; Thomas *et al.*, 2007), as well as effects that are not seen by most animals such as reflection of near-infrared light (NIR: 700 to 1700nm) (Ospina-Rozo *et al.*, 2022b) and the ability to selectively reflect circularly polarised light (Thomas *et al.*, 2007; Finlayson *et al.*, 2017; Hernández-Jiménez *et al.*, 2014; Järrendahl & Arwin, 2018). Although there have been attempts to understand the biological significance of these effects in some species (reviewed for beetles in Seago *et al.*, 2009; for other animals in Doucet & Meadows, 2009; and more recently Kjærsmo *et al.*, 2020; Franklin *et al.*, 2021; Kjærsmo *et al.*, 2022), we still know very little about the evolutionary drivers of diverse optical effects in beetles. Phylogenetic comparative studies are needed to identify selective pressures that shape this diversity, but such studies remain rare due to limited phylogenetic and trait information for most beetle groups.

Optical effects produced by beetle elytra can serve multiple functions, some of which are expected to result in correlations with climate. For example, darker organisms or darker species may be found in humid vegetated environments, a pattern known as Gloger's rule (Wittkopp & Beldade, 2009; Delhey, 2019). In these environments, darker colours enhance camouflage due to the dense canopy and dark soils, and the accumulation of melanin may protect against bacteria and parasites (Delhey, 2019; Cheng *et al.*, 2018; Zink & Remsen Jr, 1986; Wittkopp & Beldade, 2009). Conversely, darker organisms may be found in colder regions because absorbing solar radiation can aid warming, a pattern known as Bogert's rule or the thermal melanism hypothesis (Clusella Trullas *et al.*, 2007). Alternatively, higher reflectivity may be associated with hot, dry climates where organisms risk overheating (Clusella-Trullas & Nielsen, 2020; Munro *et al.*, 2019; Medina *et al.*, 2018; Stuart-Fox *et al.*, 2017; Forsman *et al.*, 2002). Correlations between beetles' coloration and the environment vary between species; for example in the families Coccinellidae and Chrysomelidae, some species follow

Gloger's rule (Dobzhansky, 1933; O'Neill *et al.*, 2017) while others follow Bogert's rule (O'Neill *et al.*, 2017; Kozlov *et al.*, 2022; Clusella-Trullas & Nielsen, 2020). Often, it is difficult to identify the selective pressures driving variation in optical properties because different selective pressures may produce similar ecogeographical patterns. However, it is possible to distinguish selection for thermal benefits from selection for other advantages by comparing patterns in different spectral ranges, i.e. UV-Visible from 300 to 700 nm and NIR from 700 to 1700nm (Clusella Trullas *et al.*, 2007; Nussear *et al.*, 2000; Luke, 1994; Norris, 1967). This is because absorption or reflection of NIR light, which accounts for 55% of solar radiant energy, affects heat gain but does not affect camouflage or signalling because these wavelengths are not visually perceived by animals (Stuart-Fox *et al.*, 2017; Clusella Trullas *et al.*, 2007). A recent phylogenetic comparative analysis in Buprestidae considered the two spectral bands separately and showed that visible reflectance varies as predicted by Gloger's rule in small individuals, while NIR reflectance is correlated with size (Wang *et al.*, 2022a). However, there are still very few studies using a comparative approach to test the biological function of diverse optical effects (But see phylogenetic comparisons for iridescence, Eliason *et al.*, 2020; Gruson *et al.*, 2019b; blue, Babarovic *et al.*, 2019; white, Stuart-Fox *et al.*, 2018; super black, McCoy & Prum, 2019; U.V. reflection, Kemp *et al.*, 2005; and 3D photonic crystals, Seago *et al.*, 2019).

Beetles use a variety of light manipulation mechanisms often combining pigments and structures. Some of these mechanisms may be phylogenetically constrained (Seago *et al.*, 2009; Sun & Bhushan, 2012) and in many cases, interactions between structures and pigments are expected (Shawkey & D'Alba, 2017), but little is known about how ecogeographical rules shape these complex optical effects. Scarab beetles (Scarabaeidae) are known for reflecting circularly polarised light, for which they use chitin fibres that arrange in spiral structures, known as Bouligand or plywood arrays or chiral arrays (Finlayson *et al.*, 2017; Sharma *et al.*, 2009; Sharma *et al.*, 2014; Arwin *et al.*, 2012). However, these chiral structures are present in only 8 out of the 15 subfamilies of scarabs and species vary in their degree of polarisation (Pye, 2010; Bagge *et al.*, 2020; Goldstein, 2006), defined as the proportion of light waves of left or right-handed circular polarisation reflected by the structure (Marshall *et al.*, 2019). Although linearly polarised light is detected and used by many animals, this is not the case for circularly polarised light (Roberts *et al.*, 2011; Temple *et al.*, 2021; Zeil *et al.*, 2014; Marshall *et al.*, 2019). Apart from some species of mantis shrimp, no other organism (neither beetles themselves nor their predators) has been shown to have a visual system capable of decoding the circular polarisation of light (Marshall *et al.*,

2019; Chiou *et al.*, 2008). Why, then, is there so much variation in the degree of circular polarisation among scarabs? One possibility is that polarisation is a secondary consequence of selection for other properties of the elytra. A chiral array could provide enhanced mechanical properties and water repellence (Mitov, 2017; Zaheri *et al.*, 2018; Yang *et al.*, 2010; Sun & Bhushan, 2012), suggesting that polarised cuticles may be more likely to occur in humid and harsh environments. Highly regular chiral structures tend to produce highly saturated iridescent colours resulting in relatively low reflectance (McDonald *et al.*, 2017; Berthier, 2007). However, in some species a chiral array is combined with disorder and additional elements to produce broadband reflectance (characteristic from gold and silver beetles: Finlayson *et al.*, 2017; Mendoza-Galvan *et al.*, 2018; Mendoza-Galván *et al.*, 2019; Vargas *et al.*, 2018; Parker *et al.*, 1998). Thus, polarisation could be negatively or positively correlated with environmental variables, but it remains unclear whether there are consistent evolutionary drivers of polarisation *per se*, or whether correlations arise due to the association between polarisation and other optical properties.

In this study, we tested for associations between optical effects and ecological variables (climate and vegetation) across species within an Australian endemic group of scarabs commonly known as Christmas beetles (Scarabeidae: Rutelinae). Christmas beetles have diverse colours and optical effects including iridescent colours, metallic gold, pearlescent appearance, diffuse brown or green, as well as iridescent and diffuse NIR reflectance (Ospina-Rozo *et al.*, 2022b). Species with highly saturated iridescent and metallic appearance reflect circularly polarised light, likely produced by simple or composite chiral arrays respectively (Ospina-Rozo *et al.*, 2022b), while bright non-polarised greens are likely produced by non-chiral composite materials which include pigment-based filters (Ospina-Rozo *et al.*, 2022a) (**Figure D-2**). Less is known about the mechanisms in NIR, but it is expected that the structures that produce iridescent NIR reflectance for some species (Ospina-Rozo *et al.*, 2022b) differ from the disordered structures that produce diffuse NIR reflectance in others (Ospina-Rozo *et al.*, 2022a). The percentage of light reflected, transmitted and absorbed by the elytra differs between species (Ospina-Rozo *et al.*, 2022c). In general, adult Christmas beetles are active only during the warmest months, i.e. December through January (Hangay & Zborowski, 2010) and are found in environments with a wide range of humidity, radiation and temperature (CSIRO Bureau of Meteorology, 2014; Westgate *et al.*, 2022) (**Figure D-1**). Due to their diversity and wide distributional range, Christmas beetles are an ideal group to explore the possible ecological correlates of different optical properties of beetle elytra.

We tested whether reflectivity and polarisation of 46 species of Christmas beetles are associated with environmental variables such as temperature, radiation, humidity, and vegetation cover, after correcting for phylogenetic relatedness. To help distinguish the importance of different selective pressures, particularly the role of selection for thermoregulatory benefits, we considered reflectivity in three different wavelength ranges visible: 400 to 700 nm, NIR: 700 to 1700 nm and total: 400 to 1700 nm. For a subset of species, we evaluated the variability in absorptivity because our recent study showed that the proportion of light absorbed by the beetle elytra is a better predictor of the heating of the elytra than reflectance. Whether this corresponds to variation in thermoregulation remains unclear since the heat of the elytra may be directly transferred to the body or dissipated by additional physiological or behavioural mechanisms (Ospina-Rozo *et al.*, 2022c). Finally, we tested for correlations between the degree of polarisation and total reflectance as well as ecological variables. We included body size as a covariate and also tested its correlation with climate because it can modulate effects of reflectance and absorbance (Clusella Trullas *et al.*, 2007) and it is often shaped by climate variables (Chown & Gaston, 2010). We show how the compilation of ecological variables and phylogenetic history of multiple species with different mechanisms of producing structural colour can be used to inform species selection in biophysics and biophotonics studies interested in the function of structural colours.

5.3 | Methods

5.3.1 | Specimen selection

We analysed 261 specimens representing 53 morphs of 46 species of the subfamily Rutelinae and one species of the subfamily Melolonthinae due to their similar locations and appearance (details in **Table D-1** and interactive table in our code). These museum specimens were provided by the Australian National Insect Collection (ANIC) and were selected to accurately represent the diversity of optical effects in this group of beetles as well as the extent of their geographical distribution.

5.3.2 | Reflectivity and Absorptivity

Based on spectrometry measurements we calculated reflectivity of 261 individuals (46 species) as the ratio of total light reflected by the elytra to the total incident radiation

(solar irradiance spectrum), integrated over the wavelength range of interest (%) (Smith *et al.*, 2016b; Ospina-Rozo *et al.*, 2022b; Ospina-Rozo *et al.*, 2022c). We calculated reflectivity for three spectral bands: visible (VIS, 400-700 nm), near infrared (NIR, 700-1700 nm) and total (Total, 400-1700 nm). We obtained the raw values of the hemispherical reflectance spectra of the beetle elytra while still attached to the beetle body with an integrating sphere containing an inbuilt tungsten-halogen light source (400-2100 nm; ISP-REF; Ocean Optics Inc., Dunedin, FL, USA.) and 4 mm diameter sampling port. We did not measure ultraviolet reflectance due to limitations of the inbuilt light source within the integrating sphere, but this should have minimal effects on our conclusions given ultraviolet wavelengths contribute <7% of the energy in solar radiation and Christmas beetles have low reflectance below 300 nm (Ospina-Rozo *et al.*, 2022b). The integrating sphere was connected to two spectrophotometers (Ocean Optics Inc., Dunedin, FL, USA), a USB 2000+ (400-1000 nm) and NIRQuest (1000-2100 nm) via a bifurcated optic fibre. Measurements were recorded using the software OceanView 1.6.7. and calibrated against a diffuse 99% reflectance spectralon standard (Labsphere, North Sutton, NH, USA).

As our previous work showed that absorptivity is a strong predictor of the heating of elytra than reflectivity (Ospina-Rozo *et al.*, 2022c), we examined the correlation between climate and absorptivity. We used the data from Ospina-Rozo *et al.* (2022c) which describes total percentage of sunlight absorbed by the elytra of a subset of 56 of the individuals (28 species) used in our larger comparative analysis. In Ospina-Rozo *et al.* (2022c) absorptivity (A) was calculated following **Equation 5-1**, as the inverse of the sum of transmissivity (T) and reflectivity (R), where the last two are standardized by the solar irradiance for the three different spectral bands.

$$A = 100 - (T + R)$$

Equation 5-1

5.3.3 | Polarisation

We used a modified digital SLR camera (D7200; Nikon Inc., Tokyo, Japan) with a UV-visible-NIR transmissive lens (60 mm Apo Macro, CoastalOpt; JenOptik, Jena, Germany) and optical filters to limit transmission to the visible range (84754, 84727 - Edmund Optics, New Jersey, USA) to take calibrated photographs for each specimen (details in Munro *et al.*, 2019). In each photo, we included a scale bar and a 40% achromatic reflectance standard (LabSphere, New Hampshire, USA) for calibration. We used a

modified flash unit (Nikon SB-140 UVIR clone; BeyondVisible, USA) as a light source. A cylindrical diffuser was placed over the sample to ensure even illumination. For each specimen we took three consecutive photos: one under the visible filter only, one under the visible filter coupled to a left-handed circular polarizer (LCP, CP42HE - Edmund Optics, New Jersey, USA) and one under the visible filter coupled to a right-handed circular polarizer (RCP, CP42HER - Edmund Optics, New Jersey, USA). Thus, when we mention polarisation, we are only referring to circular polarisation in the visible range. We used magnetic rings to attach the polarizers to the visible filter of the camera to easily alternate between the three configurations without altering any other components in the setup, enabling the alignment of the three pictures for each beetle. We photographed a set of achromatic standards with known reflectance equivalent to 2%, 20%, 40%, 60%, 80% and 99% for each configuration of the camera and lights on each day to enable accurate calibration (**Figure D-3**).

To obtain a measurement of the amount of light reflected for each polarisation we extracted RGB values for two different regions of interest (ROI) for each beetle, one on the elytra and one on the pronotum since these two structures may be under different selective pressures. We derived an estimated of reflectance from the RGB values of one ROI on the surface of the beetle that was not shaded and had no glare spot. The two polarization photographs were aligned so that the same ROI was selected in both cases. For each ROI we obtained the mean pixel intensity values for red (R), green (G), blue (B) channels and the achromatic standard using the MeasureRGB plugin in ImageJ (v. 1.52a; Schneider *et al.*, 2012). Afterwards, the raw RGB values in each colour channel were transformed to their correspondent reflectance with an equation derived from the set of known achromatic standards. Then, the values were equalized to the grey standard in each photo to make the information from all pictures comparable (Munro *et al.*, 2019; Wang *et al.*, 2022a). For details on this process see **Figure D-3** and our code. Finally, we averaged the calibrated values to give an estimate of average reflectance for each ROI in each polarization.

In this work, we considered reflectance in the LCP filter a measurement of the light manipulated by chiral structures, and reflectance in RCP filter a coarse indicator of the accumulation of pigments. This is because the chiral nanostructures in most scarabs selectively reflect left-handed circular polarised light (Sharma *et al.*, 2014; Mitov, 2017). Thus, LCP filters capture the optical effects associated to these structures and RCP “cancel” them. The remaining reflectance in RCP filters likely corresponds to light interacting with deeper layers of pigment. For example, Christmas

beetles showed similar optical effects in the visible and LCP filters, while most varied between light brown and black in the RCP filter (Figure 5-1). These two colours are usually associated with the presence of melanin in the beetles' cuticle (Noh *et al.*, 2016; Seago *et al.*, 2009; Futahashi & Osanai-Futahashi, 2021).

We used the degree of circular polarisation (Equation 5-2) as a standardised measurement of left-handed polarised light:

$$P = \frac{R_l - R_r}{R_l + R_r}$$

Equation 5-2

A larger value in the degree of polarisation indicates larger difference in RGB values between the LCP and RCP filters, thus a greater proportion of light manipulated by the left-handed chiral structures.

We considered the average RGB values in the RCP filter inversely proportional to the accumulation of pigments, likely melanin. Although chiral structures that reflect right-handed circularly polarised light exist in nature, very few species have developed the structural modifications required for this effect (Bagge *et al.*, 2020; Finlayson *et al.*, 2017). In addition, melanins are large, biochemically stable polymers that crosslink with proteins in the beetle cuticle, so their associated colours are unlikely to significantly fade under controlled museum conditions such as low exposure to light, heat, oxygen, humidity, acidity and bacteria (Doucet & Hill, 2009; McNamara *et al.*, 2012; McNamara & Orr, 2013; Noh *et al.*, 2016). Thus, our simple approach to consider all the light reflected in the RCP filter as pigment-related is still valid to study the interplay between pigments and structures in this group of beetles.

5.3.4 | Body size

From the calibrated photographs taken in the previous step we obtained the length of each beetle based on the reference scale in ImageJ (v. 1.52a; Schneider *et al.*, 2012). This measurement is a good proxy for body size in Christmas beetles since their shape is very conserved.

5.3.5 | Climate data

We collected climate data for each specimen locality during the months of peak activity for the correspondent species. We determined the months of peak activity for each species based on the collection records reported in the Atlas of Living Australia (ALA, 2021): for each species we calculated the percentage of records for each month relative to the total, and only considered the months with a number of records equivalent to >10% of total records for the species. Then, we extracted climate data for the global positioning system (GPS) coordinates of the museum labels of our samples.

We obtained information on climatic variables from continent-wide 0.05° grids of interpolated weather data from the Australian Gridded Climate Data (AGCD)/AWAP database (Australian Bureau of Meteorology official dataset for monthly gridded rainfall analysis) averaged over 10 years (2010 - 2020) considering only the interval of the activity peak for each species. The climate variables were number of extreme temperature days (maximum temperature > 35°C); daily measurements averaged per month of the average maximum temperature (°C), average minimum temperature(°C), average solar radiation ($\text{kJ} \times \text{m}^{-2} \times \text{day}^{-1}$) and rainfall (mm/day); monthly mean of the vapor pressure (hPa) and yearly mean of cloud cover Cloud coverage (%) was calculated from the ratio of observed integrated daily solar radiation to that of a cloudless day. In addition, we obtained the following variables related to vegetation coverage from the Atlas of Living Australia (Westgate *et al.*, 2022): Mean annual net primary productivity (tonnes/ha/yr), fractional cover of bare soil (2012-03-05), leaf area index (LAI), fraction of Photosynthetically Active Radiation (fPar) in the spectral range 400-700nm used by plants for photosynthesis, mean annual aridity index (here multiplied by -1 to facilitate its interpretation since it is originally calculated as the monthly ratio of precipitation to potential evaporation), mean annual growth index C3 macrotherm plants, mean annual growth index C3 mesotherm plants and mean annual growth index C4 megatherm plants.

Due to the multivariate nature of the climate, we used a principal component analysis (PCA) as a variable reduction tool (Hurtado *et al.*, 2020; Medina *et al.*, 2018). To minimise redundancy, we examined the pairwise correlations between variables, and kept only 7 ecological variables that were effective to represent the differences between samples and did not have correlations with Pearson's $R^2 > 0.8$ (Table D-2). All vegetation variables correlated strongly to rain and vapor pressure, while maximum and minimum temperature were also strongly correlated. Thus, the 7 variables

included in the PCA were maximum temperature, number of days above 35°C, solar radiation, cloud cover, rain, water vapour and aridity.

We retained only the first two climate principal components (PCs) since they had eigen values >1 (**Figure D-4**) and together explained a total of 82% of the variability in the data. PC1 explained 42.3% of the total climate variation and correlated negatively with vapor pressure, rain, cloud cover and temperature; while it correlated positively with aridity. PC2 explained 39.5% and correlated negatively with solar radiation, number of days over 35°C, temperature and aridity, while simultaneously correlating positively with cloud cover. Overall, lower PC1 values represented humid environments with more cloud cover and vegetation coverage, while lower PC2 values represented hotter, more arid environments with more solar radiation (**Figure D-4**).

5.3.6 | Phylogenetic inference

We compiled a species-level supermatrix of genetic data using i) the COI data of Mitchell (2015) and ii) additional framework and data from Gunter et al. (2016), via sequence data downloaded from NCBI (<https://www.ncbi.nlm.nih.gov/>). Andrew Mitchell and Chris Reid (pers. comm.) provided taxonomic and sample identity advice. This resulted in a final datamatrix of three genes (28s, 16s, COI) (MCC tree in **Figure D-5**) then subjected to Bayesian analysis (BEAST; Bouckaert et al. 2014) providing 1300 posterior sample trees to represent the phylogenetic information accounting for uncertainty in node ages and topology.

5.3.7 | Phylogenetic comparative analysis and correlations

We used a combination of phylogenetically controlled models to evaluate if three optical properties in the Christmas beetles' elytra - reflectivity, polarisation, and absorptivity - can be predicted by climate and size. Although all our variables were measured on multiple individuals per species, it was not our aim to test if climate or size can predict the intraspecific variability in optical properties. Instead, we averaged the data for each variable per species and conducted our analysis at the species level. We used a PGLS (phylogenetic generalised least squares) model on the MCC tree and accounted for phylogenetic uncertainty by also fitting the PGLS model to a subset of 1001 trees from the posterior distribution. In some cases, models could not be fitted to a small number of the trees due to convergence issues associated with those particular trees; however, all models were fitted to >960 trees (see **Table D-3** for

precise number of trees for each model). To test the amount of phylogenetic signal in the optical properties: total, visible, NIR reflectivity, absorptivity and polarisation, we used Pagel's lambda method, with higher values (closer to 1) representing traits with high phylogenetic signal (detailed results in **Table D-4**).

For the models with reflectivity and absorptivity as response variables we ran three separate phylogenetically controlled models (PGLS), one for each reflectance range: visible (VIS), near-infrared (NIR) and total (the sum of the previous two), considering as predictors two climatic principal components (PC1 and PC2), body size, and the interactions between body size and climatic PCs. The optical properties in visible and NIR reflectance are part of a continuous spectrum, and therefore are often correlated. To account for this correlation, we added the visible reflectivity/absorptivity as a predictor when NIR was the response variable. In addition, we calculated the residuals of the linear regression between NIR and VIS while accounting for phylogenetic relatedness and used these residuals as the response variable (instead of the raw NIR values) in a separate set of models. We expected the results of these two to be consistent. To determine if the trends found by our models are strongly influenced by outliers we performed the same PGLS models excluding these outliers (results in supplementary materials). We considered *A. prasinus* and the species belonging to the genus *Xylonychus* and *Paraschizognathus* to be outliers since our data in Chapter 3 showed they have a special bi-layered photonic mechanism which enhances NIR reflectance.

To evaluate if body size correlates with climate, we ran a PGLS considering body size as the response variable and the two climatic principal components PC1 and PC2.

To test if polarization itself is correlated to any of our ecological variables or size, we ran a PGLS using the same predictors as for reflectivity: two climatic principal components (PC1 and PC2), body size, and the interactions between body size and climatic PCs. To evaluate if the degree of polarization can predict the amount of light reflected in the visible and NIR spectral bands, we also used a phylogenetic controlled model (PGLs). To determine if correlations between these variables are mainly driven by outliers, we compared the correlations between the variables in different subsets of data excluding the outliers and the full data set. One of these subsets excludes *A. prasinus*, and the species belonging to the genus *Xylonychus* and *Paraschizognathus* since data in Chapter 3 showed they have a different photonic mechanism than that expected for scarabs. The other subset excludes certain species that cluster together

and separately from remaining species (see results). We used the Pearson's correlation coefficient in these comparisons (results in supplementary materials).

All our models were run in R Statistical Software (v4.1.2 R Core Team, 2021) To run PGLS models we used the packages *ape* (Paradis & Schliep, 2019), *caper* (Orme *et al.*, 2018), *picante* (Kembel *et al.*, 2010) and *phytools* (Revell, 2012). In order to run the PGLS model in multiple trees, we used loop functions programmed in R following Medina *et al.* (2018). We report the 95% intervals for both the estimate and p value obtained from the iterations of fitting the model to multiple phylogenetic trees. To ensure that our models meet the assumptions of normality and homoscedasticity in the residuals we examined the diagnostic plots for the residuals on each case. In order to avoid binary decision making associated to the use of single p-values, for all our tests, we report the confidence intervals (Halsey, 2019) around the estimate and p-value for single tests and across trees in the case of PGLS models. We use language of gradual evidence to interpret p-value intervals (Muff *et al.*, 2022), whereby we state how much evidence our results offer to support our hypotheses according to the following approximate ranges (boundaries are not hard thresholds): Results offer little to no evidence = p-value C.I. (confidence interval) >> 0.1, weak evidence = p-value C.I. around 0.05 and 0.1, moderate evidence = p-value C.I. around 0.01 and 0.05, strong = p-value C.I. around 0.001 and 0.01, very strong evidence = p-value C.I. around 0.0001 and 0.001.

5.4 | Results

5.4.1 | Diversity in optical properties

We observed great variability in reflectivity in the three spectral bands (VIS, NIR and Total) and degree of polarization in Christmas beetles (Figure 5-1). However, these traits appear to cluster according to phylogeny, as confirmed by the high phylogenetic signal quantified by Pagel's lambda which ranged from 0.81 to 0.97. Phylogenetic signal was notably high for NIR reflectivity (0.93) and polarisation (0.95). (Table D-4). Beetles varied between light brown to black under the RCP filter which can be interpreted as a proxy for the amount of pigment accumulated in the elytra (Figure 5-1).

5.4.2 | Correlations between reflectivity, body size and climate

Our results showed opposite patterns for visible and NIR reflectivity (Table 5-1), which explains why none of our variables - PC1 (humidity), PC2 (temperature) or size - predicted total reflectivity. We found moderate evidence that visible reflectivity was predicted by the interaction between PC1 (humidity) and body size (Table 5-1): The interaction term was significant in the MCC tree ($p\text{-value} = 0.049$) but had less support after considering uncertainty in the phylogeny ($0.014 < p\text{-value} < 0.082$ across sampled trees). Smaller beetles tended to be lighter (higher visible reflectivity) in more humid environments (Figure 5-2A) while larger beetles tended to be darker in more humid environments (Figure 5-2B). There was a strong main effect of body size, with larger beetles being lighter overall ($0.008 < p\text{-value} < 0.053$; Table 5-1; Figure 5-3A). Larger beetles also occurred in drier environments with less vegetation cover (Figure 5-3B; MCC estimate = 0.088, multiple trees estimate C.I. = 0.089; 0.097, $p\text{-value}$ C.I. = 0.005; 0.021).

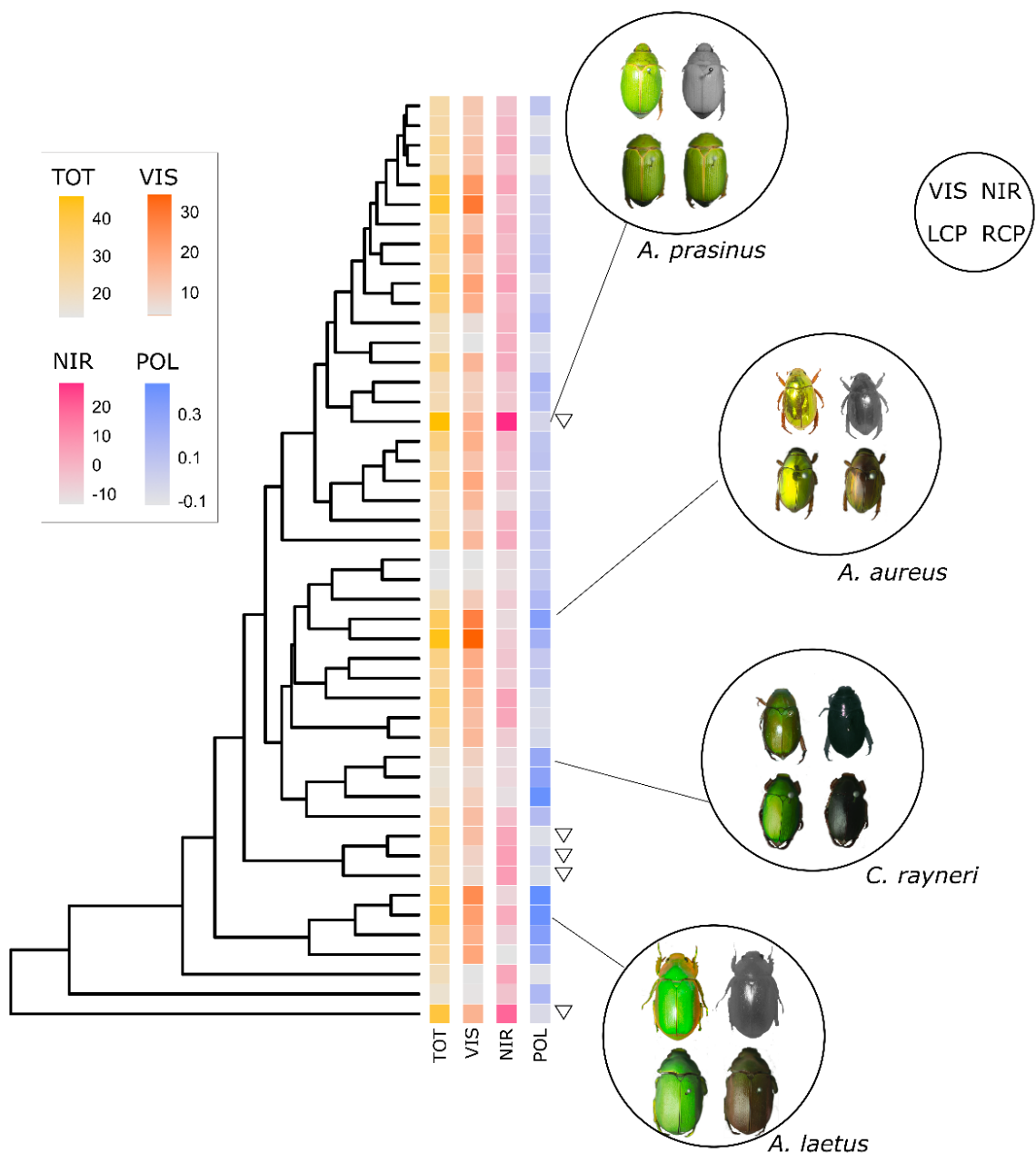


Figure 5-1. Optical properties mapped onto the MCC tree for Christmas beetles

Coloured squares (heat map) show reflectivity in three different spectral bands (VIS - visible; NIR - the residuals of a linear regression between the NIR reflectivity and VIS reflectivity; TOT - the sum of the raw visible and NIR reflectivity) and the degree of polarization (POL - the standardized difference between the reflectance in left and right-handed polarization). Triangles represent species with a white underlay (non-chiral composite structure Ospina-Rozo *et al.*, 2022a). Calibrated photographs of four species of beetles are shown under different filters: visible (VIS), near-infrared (NIR), left-handed circular polarization (LCP) and right-handed circular polarization (RCP), as an example of the diversity in optical properties.

Table 5-1. Relationships between reflectivity, environmental PCs and size.

Reflectivity was studied in the three spectral bands, visible, NIR and total. Posterior 95% confidence intervals C.I. of the regression estimates, and p-values obtained by applying the

PGLs function on >900 trees to account for phylogenetic uncertainty. The highlighted results provide evidence for correlations due to their estimate interval not overlapping with 0 and p values lower than 0.05.

Optical property	Predictor	Estimate in MCC	Estimate interval	p-value
Total reflectivity (400 to 1700nm)	PC1	-5.708	-7.355 ; -4.786	0.056 ; 0.248
	PC2	-0.927	-2.871 ; 1.017	0.605 ; 1.000
	Size	5.998	4.106 ; 7.896	0.039 ; 0.307
	PC1 x Size	2.653	2.237 ; 3.326	0.039 ; 0.221
	PC2 x Size	0.302	-0.489 ; 1.083	0.614 ; 1.000
VIS reflectivity (400 to 700nm)	PC1	-6.177 (*)	-7.737 ; -5.307	0.017 ; 0.093
	PC2	-6.831	-8.333 ; -5.344	0.029 ; 0.166
	Size	7.075 (*)	6.080 ; 8.428	0.008 ; 0.053
	PC1 x Size	2.723 (*)	2.388 ; 3.364	0.014 ; 0.082
	PC2 x Size	2.563	1.939 ; 3.163	0.041 ; 0.195
NIR reflectivity (Including VIS reflectivity as a predictor)	PC1	2.081	0.869 ; 3.144	0.404 ; 0.808
	PC2	12.647 (*)	10.375 ; 15.124	0.000 ; 0.023
	Size	-3.652	-5.348 ; -1.774	0.145 ; 0.599
	PC1 x Size	-0.691	-1.253 ; -0.152	0.439 ; 0.925
	PC2 x Size	-4.866 (*)	-5.888 ; -3.974	0.001 ; 0.026
	Visible	1.163 (*)	1.113 ; 1.219	0.000 ; 0.000
Residuals (NIR-VIS regression and corrected for phylogenetic relatedness)	PC1	1.376	0.212 ; 2.294	0.502 ; 0.940
	PC2	11.781 (*)	9.576 ; 14.173	0.000 ; 0.026
	Size	-2.772	-4.388 ; -1.084	0.207 ; 0.707
	PC1 x Size	-0.371	-0.900 ; 0.138	0.597 ; 0.999
	PC2 x Size	-4.531 (*)	-5.544 ; -3.718	0.001 ; 0.029

Note: (*) in the estimate MCC column highlights those results that had p-values ≤ 0.05 when using the MCC tree.

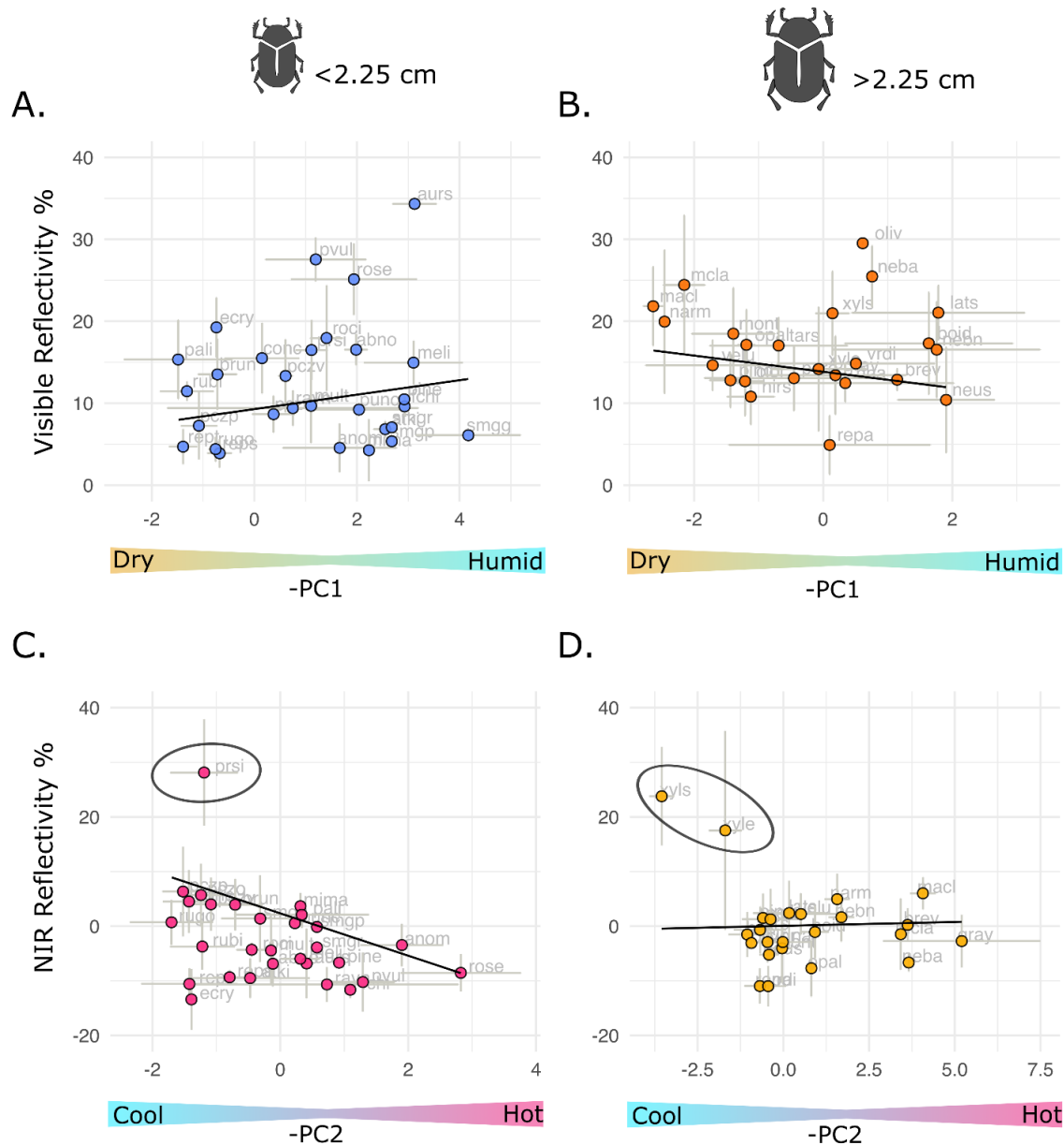


Figure 5-2. Effects of the interactions between climate and size on reflectivity

For visualisation, species are divided into those smaller and larger than the mean body size (2.25 cm). The plots show the mean and standard deviation (error bars) of the studied optical properties and PC values per species. NIR reflectivity is given as the residuals of a simple linear model with visible reflectivity as a predictor for visualisation purposes. Trend lines were calculated based on the estimates of the PGLS models (Table 5-1). PC2 was fixed at the mean value for all beetles in A and B while PC1 was fixed at the mean value for all beetles in C and D. Outlier species with very high NIR reflectivity are circled in A and C. Further PGLS analyses were run without these species to evaluate their effect in the general trends.

NIR reflectivity was associated with PC2 (temperature) rather than PC1 (humidity). Specifically, for both NIR models (NIR reflectivity as the response variable with VIS reflectivity as a predictor, and residuals of the NIR-VIS regression as the response variable) we found moderate to strong evidence for an interaction between PC2 and body size (p-values in the MCC tree were 0.009 for NIR and 0.01 for NIR residuals, intervals in Table 5-1). Contrary to Bogert's rule (thermal melanism - darker in cooler environments), species with higher NIR reflectivity occurred in cooler environments, though this pattern was most apparent for smaller species and largely driven by outliers with exceptionally high NIR reflectivity (circled in Figure 5-2C and Figure 5-2D) correspondent to *Anoplognathus prasinus* and *Xylonychus* sp. (species with a white underlay that enhances NIR reflectivity). This was confirmed by our PGLS models excluding the outliers, since when excluding the species with a white underlay reported in Ospina-Rozo *et al.* (2022a) the evidence for an interaction between PC2 and body size decreased considerably (p-values in the MCC tree were 0.163 for NIR and 0.094 for NIR residuals, p-value C.I. = 0.065 ; 0.394 for NIR and 0.032 ; 0.257 for NIR residuals, Table D-5).

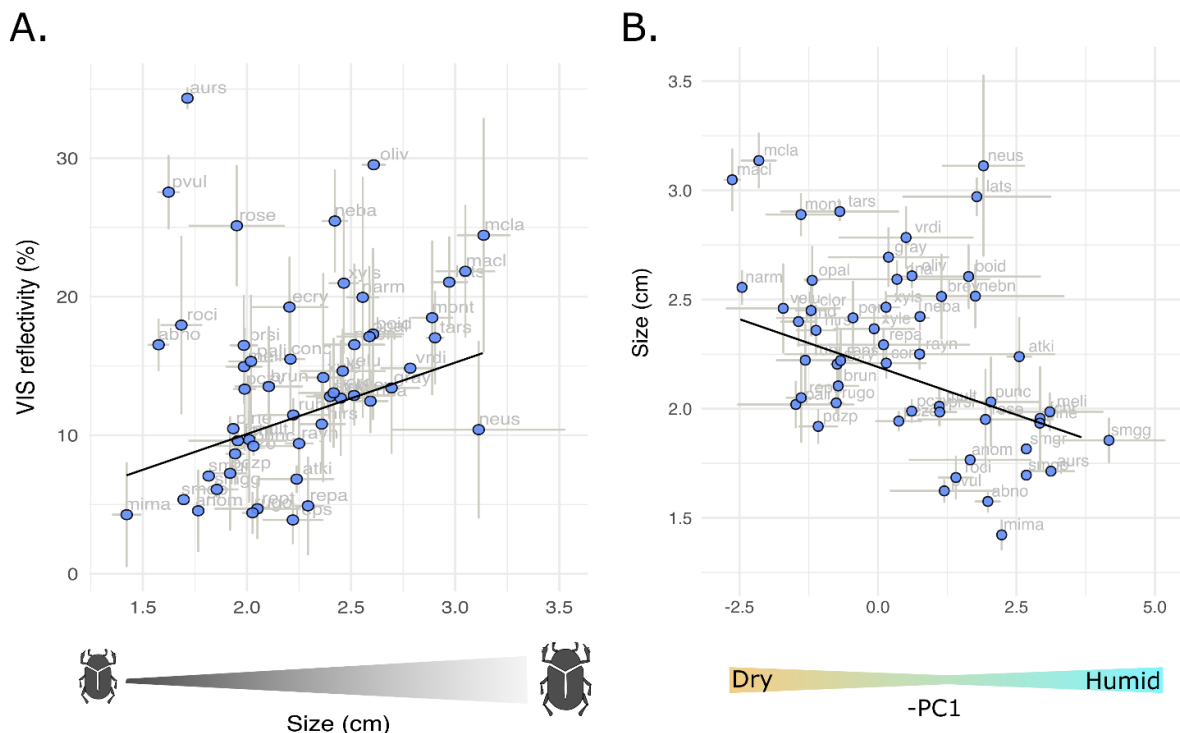


Figure 5-3. The importance of body size

Larger beetles reflect more visible light and occur in drier environments with less vegetation cover. The plots show the mean and standard deviation (error bars) for each variable. Trend lines were calculated based on the estimates of the PGLS models.

5.4.3 | Correlations between absorptivity, body size and climate

Our results showed only moderate evidence for a negative correlation between NIR absorptivity and PC2 (-Temperature) suggesting that beetles living in cooler environments with lower temperatures absorb less NIR light. (Table 5-2), which is consistent with the results for NIR reflectivity (beetles in cooler environments reflect more NIR light). This pattern, however, also disappears after excluding the species with high NIR reflectivity (Table D-5). In addition, we did not find any qualitative evidence to support the hypothesis that beetles living in hotter environments can implement both low absorptivity (as a result of high reflectivity), and high absorptivity combined with extra mechanisms to dissipate heat because we did not observe a parabolic relationship between absorptivity and PC2 (Figure D-6 and Figure D-7).

Table 5-2. Relationships between absorptivity, environmental PCs and size.

Posterior 95% confidence intervals C.I. of the regression estimates, and p-values obtained by applying the PGLs function on >900 trees to account for phylogenetic uncertainty. The highlighted results provide evidence for correlations.

Optical Property	Predictor	Estimate in MCC	Estimate interval	p-value
Total absorptivity	PC1	6.728	1.207 ; 6.748	0.508 ; 0.917
	PC2	-3.479	-5.112 ; 1.470	0.69 ; 1.000
	Size	4.856	0.134 ; 4.856	0.607 ; 0.970
	PC1 x Size	-3.289	-3.293 ; -0.691	0.475 ; 0.877
	PC2 x Size	0.658	-1.343 ; 1.117	0.779 ; 0.998
VIS absorptivity	PC1	3.44	1.660 ; 6.212	0.430 ; 0.821
	PC2	5.606	1.332 ; 6.826	0.474 ; 0.890
	Size	-1.656	-3.988 ; 0.345	0.607 ; 0.964
	PC1 x Size	-1.644	-2.957 ; -0.855	0.418 ; 0.803
	PC2 x Size	-2.346	-2.929 ; -0.907	0.437 ; 0.815
NIR absorptivity	PC1	-1.563	-2.201 ; -0.533	0.627 ; 0.910
	PC2	-11.891 (*)	-14.043 ; -10.650	0.016 ; 0.078
	Size	8.861	7.569 ; 9.276	0.049 ; 0.110
	PC1 x Size	0.616	0.178 ; 0.932	0.639 ; 0.920
	PC2 x Size	4.334	3.783 ; 5.054	0.029 ; 0.113
	Visible	1.354 (*)	1.296 ; 1.363	0.000 ; 0.000
Residuals absorptivity	PC1	-1.532	-2.271 ; -0.739	0.593 ; 0.863
	PC2	-11.861 (*)	-14.052 ; -10.647	0.012 ; 0.068
	Size	8.854	7.664 ; 9.294	0.041 ; 0.098
	PC1 x Size	0.601	0.263 ; 0.987	0.620 ; 0.896
	PC2 x Size	4.321	3.823 ; 5.086	0.020 ; 0.098

Note: (*) in the estimate MCC column highlights those results that had p-values ≤ 0.05 in the MCC tree.

5.4.4 | Correlations between polarisation, climate and optical properties

The degree of polarisation was not predicted by climate or size (Table 5-3) and it does not vary considerably between the pronotum and elytron within the same individual (Paired t test, $t = -1.2468$, $df = 173$, $p\text{-value} = 0.2142$), however it is correlated with reflectivity and absorptivity in the NIR (Table 5-3). In this work, the degree of polarisation was interpreted as the proportion of light manipulated by the left-handed chiral structures while the RGB values in the RCP filter were interpreted as a proxy for the presence of pigments in the cuticle. According to our results, reflectivity in the visible spectral range is primarily correlated with the presence of pigments and not with chiral nanostructures (Table 5-3). This is because there was a correlation between visible reflectivity and the RGB in the RCP filter (Figure 5-4) but there was no correlation with the degree of polarisation (Figure 5-5). Lower reflectivity and high absorptivity of NIR were dependent on the presence of pigments and chiral structures since NIR reflectivity was negatively correlated with the degree of polarisation and positively correlated with the mean RGB of the RCP filter (Figure 5-5 and Table 5-3).

Table 5-3. Associations between polarisation and 1) environmental PCs and size, 2) reflectivity and 3) absorptivity

Posterior 95% confidence intervals C.I. of the regression estimates, and p-values obtained by applying the PGLs function on >900 trees to account for phylogenetic uncertainty. The highlighted results provide evidence for correlations.

Correlations	Variable	Estimate in MCC	Estimate interval	p-value
DegPol ~ Climate	PC1	-0.012	-0.026 ; 0.008	0.633 ; 1000
	PC2	-0.088	-0.133 ; -0.057	0.032 ; 0.307
	Size	0.012	-0.002 ; 0.046	0.443 ; 0.995
	PC1 x Size	-0.004	-0.013 ; 0.002	0.614 ; 1000
	PC2 x Size	0.030	0.016 ; 0.046	0.061 ; 0.441
Reflectivity ~ DegPol	VIS	8.5383	1.784 ; 12.115	0.123 ; 0.786
	NIR	-28.46 (*)	-31.732 ; -26.992	<0.001 ; <0.001
	NIR residuals	-27.89 (*)	-30.036 ; -26.445	<0.001 ; 0.002
Absorptivity ~ DegPol	VIS	5.99	0.507 ; 12.633	0.420 ; 0.979
	NIR	18.596 (·)	17.187 ; 20.929	0.002 ; 0.007
	NIR residuals	18.595 (·)	17.241 ; 20.954	0.023 ; 0.071
Reflectivity ~ RCP RGB	VIS	2.488 (*)	2.325 ; 2.675	<0.001 ; <0.001
	NIR	2.138 (*)	1.569 ; 2.343	0.004 ; 0.028
	NIR residuals	1.092 (·)	0.861 ; 1.385	0.021 ; 0.132
Absorptivity ~ RCP RGB	VIS	-0.520	-1.11 ; -0.097	0.419 ; 0.914
	NIR	0.511	0.173 ; 0.707	0.385 ; 0.863
	NIR residuals	0.507	0.175 ; 0.671	0.384 ; 0.848

Note: “DegPol” refers to degree of polarisation (see Equation 5-2) which is proportional to the amount of light manipulated by left-handed chiral nanostructures. “RCP RGB” refers to the RGB values measured under the right-handed polarised filter, which is inversely proportional to the amount of pigment. For both reflectivity and absorptivity, the notation “NIR” refers to the model with NIR as the response variable and polarisation and VIS as predictors; the notation “residuals” refers to the model with the residuals of the regression between visible and NIR the response variable and polarisation as the only predictor. (*) in the estimate MCC column highlights those results that had p-values ≤ 0.05 in the MCC tree and (·) was used for p-values in the interval $0.05 < \text{p-value} < 0.1$.

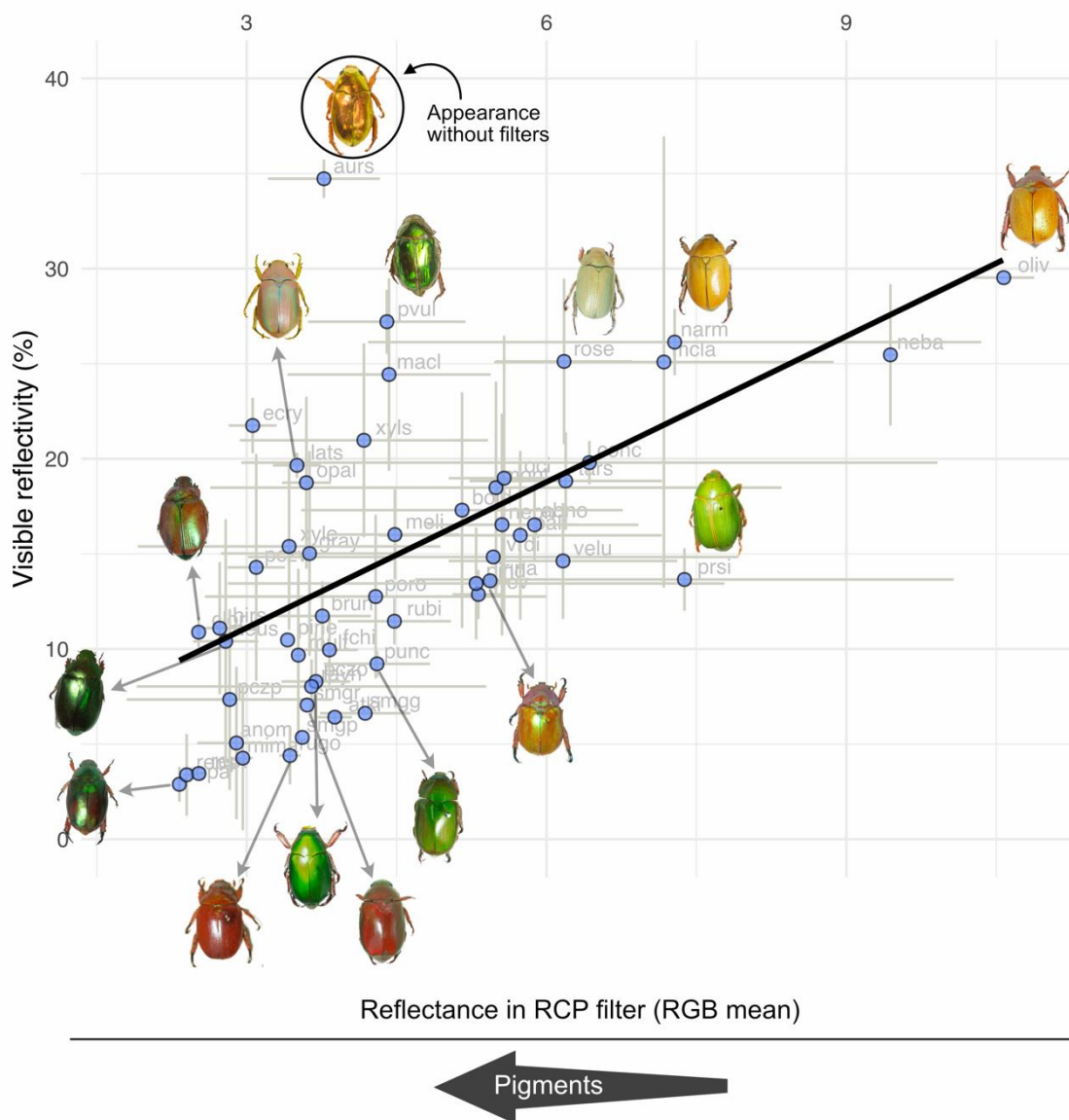


Figure 5-4. Correlation between potential pigment accumulation and visible reflectivity

Here we consider the mean RGB values under the RCP filter to be inversely proportional to the amount of pigment in the elytra. Beetles that are darker when photographed under the RCP filter potentially accumulate more pigment and have lower reflectivity, yet their overall appearance (no filter) remains diverse due to the effects created by the chiral nanostructures. Beetles are not scaled proportionally to their original size. The plot shows the mean and standard deviation (error bars) of the two variables per species. The trend line was calculated based on the estimates of the PGLS models (Table 5-3).

The correlations between polarisation and additional optical properties should be interpreted with caution. These correlations may be driven by a few extreme values that cluster and deviate from the general pattern and they may depend on the underlying mechanisms as seen by the enhanced NIR reflectivity of species with white underlays (squares in figure 4. Described in Ospina-Rozo *et al.*, 2022a). To account for these scenarios, we created three subsets of species: 1) one group excluding the species that cluster in Figure 5-5 (circled) namely *A. aureus*, *A. parvulus*, *A. roseus*, *A. laetus*, *A. opalinus* and *E. lamprimoides*, (**Figure D-8**); 2) a group excluding the species with white underlays (squares in Figure 5-5) namely *Xylonichus* sp., *A. prasinus*, *P. olivaceous*, *P. ocularis* and *P. prasinus* (**Figure D-9**); and 3) a group excluding the 11 species mentioned previously. Then, we calculated Pearson's correlation coefficient for the same correlations presented in Table 5-3 for the full set and the three subsets and compared the results to determine if the patterns were driven mainly by extreme values (**Table D-6**). Our analysis confirmed that the negative correlation between polarisation and NIR reflectivity is not driven by extreme data points. The patterns for absorptivity are not as robust as the ones for reflectivity probably due to a smaller data set. That is, the positive correlation between polarisation and the raw values of NIR absorptivity remains even after excluding extreme values, but it disappears after the same procedure with NIR residuals. The species that cluster in Figure 5-5 (circled) seem to follow unusual patterns since they produce higher VIS and NIR reflectivity and lower VIS and NIR absorptivity than the other species studied, despite having high degrees of polarisation.

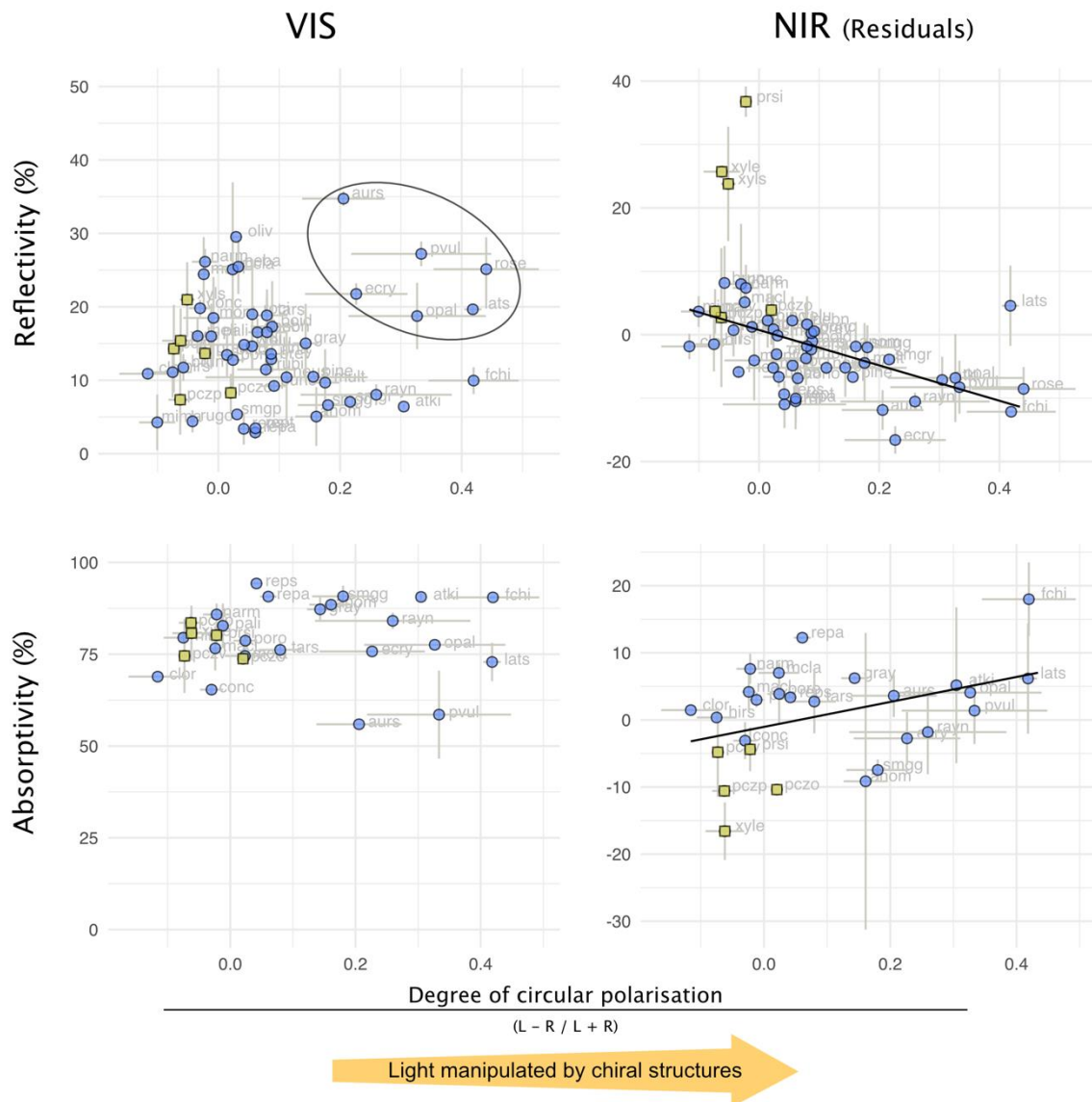


Figure 5-5. Correlations between the degree of polarization and beetles' reflectivity and absorptivity in visible and NIR

Here we consider higher degree of circular polarisation as an indication of more light manipulated by chiral structures. Beetles with a higher degree of circular polarisation absorb more and reflect less NIR, but the degree of circular polarisation is not correlated to reflectivity or absorptivity in visible light. Species with a white underlay, i.e. non-chiral structure (squares) have very high NIR reflectivity. Some species seem to deviate from the general pattern (circled). Plots show the mean and standard deviation (error bars) of the studied optical properties per species. The NIR reflectivity and absorptivity are given as the residuals of simple linear models with the visible reflectivity and absorptivity (respectively) as predictors for visualisation purposes. Trend lines were calculated based on the estimates of the PGLS models (Table 5-3).

5.5 | Discussion

The striking optical effects produced by Christmas beetles do not follow one universal ecogeographical pattern. Instead, the complex photonic mechanisms used by these beetles to reflect and absorb light, seem to give them enough flexibility to “go against the rules”. For example, visible and relative NIR reflectivity follow opposite patterns, so there is no simple relationship between ecological variables and total reflectance. Larger beetles are lighter (high visible reflectivity) and occur in drier environments, but neither size nor humidity affect relative NIR reflectivity. Larger beetles follow Gloger’s rule (darker in more humid environments); yet small beetles seem to follow the opposite pattern. Contrary to Bogert’s rule (thermal melanism - darker in colder environments), species with higher NIR reflectivity and lower NIR absorptivity occurred in cooler environments, though this pattern was most apparent for smaller species and largely driven by outliers with exceptionally high NIR reflectivity produced by a white underlay layer below the cuticle. We did not find any evidence of a correlation between polarisation and climate nor polarisation and visible reflectivity. Nevertheless, our results showed that the accumulation of pigment in the elytra (coarsely measured) can reduce both visible and NIR reflectivity, but the presence of chiral structures (measured as the degree of polarisation) reduces only the NIR reflectivity. Thus, our results suggest that the interplay between structures and pigments can create complex patterns of geographical variation. Even though reflectivity, absorptivity and polarisation are constrained by phylogeny, the patterns we detected could inform future studies on the function of light manipulation in beetles.

Ecogeographical rules to explain inter and intraspecific colour variation have been formulated mainly based on the properties of pigment-based colours - mostly melanin, thus, they may not accurately explain variations in structural colour. Melanin is a broadband absorbing pigment with multiple functions (Cordero & Casadevall, 2020; Solano, 2014) that can facilitate absorption of radiative energy (Bogert’s rule), protect against pathogens and predators (Gloger’s rule), prevent desiccation (melanin-desiccation hypothesis) and absorb harmful ultraviolet light (ultraviolet protection hypothesis) (Clusella Trullas *et al.*, 2007; Clusella-Trullas & Nielsen, 2020). However, for organisms that use structural colour, measurements of darkness or lightness may not correspond directly with the content of melanin (Delhey, 2019). The overall optical effects that correlate to ecological variables are produced by different interactions between pigments and structures (Shawkey & D’Alba, 2017). For example,

birds produce dark and bright colours with melanin (Norden *et al.*, 2019; Eliason *et al.*, 2016), insect nanostructures can enhance reflection of solar radiation (silver ants hairs, Shi *et al.*, 2015) or trap light to absorb energy (green and white butterflies, Han *et al.*, 2013; Shanks *et al.*, 2015), and chameleons have different layers of structures to manipulate visible and NIR light separately (Teyssier *et al.*, 2015). Christmas beetles seem to combine pigment and structural colours in their elytra, which could enable more flexibility to manipulate light in NIR and visible spectral bands. It could also allow them to obtain some benefits associated with melanin without becoming visibly darker or achieve high absorptivity with structural mechanisms. Thus, opposite patterns for the correlations in NIR and visible light bands, and complex correlations with polarisation, size and climate may be the result of species-specific combinations of selective pressures driving the evolution of both structures and pigments.

Water balance is a good candidate for a selective pressure driving variation in body size and visible reflectivity in Christmas beetles. We found a negative correlation between size and humidity, which is expected in insects (converse Bergmann's rule, Chown & Gaston, 2010; Mousseau, 1997) because larger individuals can resist resource shortages, and lose less water due to their small surface area : volume ratio and thicker boundary layers (Bartholomew & Heinrich, 1978; Harris *et al.*, 2012; Porter & Gates, 1969). Similar results have been found in grasshoppers and seed-feeding beetles (Bidau & Martí, 2007; Harris *et al.*, 2012; Stillwell *et al.*, 2007). Larger beetles living in dry environments were also lighter. Increased reflectivity in dry environments may be a consequence of limited food sources since melanin production in insects is costly and affected by diet (Stoehr, 2006; Evison *et al.*, 2017). Alternatively, it may counteract thermal disadvantages of large body size due to lower evaporative heat loss and greater thermal inertia (Pincebourde *et al.*, 2021; Porter & Gates, 1969; Rozen-Rechels *et al.*, 2019; Stevenson, 1985). Finally, higher reflectivity in dry environments may be associated with camouflage because substrate colour is often lighter in drier environments (Rommel & Tammaru, 2009; Cheng *et al.*, 2018; Stelbrink *et al.*, 2019; Delhey, 2018; Mänd *et al.*, 2007). Not all beetles follow the same patterns: small jewel beetles follow Gloger's rule (Wang *et al.*, 2022a), but small Christmas beetles follow the opposite trend (thought only supported by moderate evidence). Jewel beetles are active in spring and perch on flowers, while Christmas beetles are active in summer and are ground dwellers or perch in eucalyptus trees (Slipinski & Lawrence, 2019). Thus, small Christmas beetles are at stronger risk of desiccation and may accumulate melanin to resist it (Parkash, 2010; Parkash *et al.*, 2009; Clusella-Trullas & Nielsen, 2020; Evison *et al.*, 2017; Caglar *et al.*, 2013) becoming darker in drier environments.

Other studies have highlighted that, for insects, water availability can sometimes have a stronger effect on body size and colouration than thermal challenges (Chown *et al.*, 2011; Clusella-Trullas *et al.*, 2011) and our findings support this claim.

Our results suggest that thermoregulation is not a strong selective pressure driving interspecific variation in reflectivity and absorptivity. Although reflectivity affects heat gain of elytra in Christmas beetles (Ospina-Rozo *et al.*, 2022c), we did not find a strong correlation between relative NIR or total reflectivity and temperature. These results differ from other studies demonstrating that thermoregulation is associated with colour variation in birds (Dalrymple *et al.*, 2008; Medina *et al.*, 2018; Galván *et al.*, 2018), heliothermic lizards (Clusella-Trullas *et al.*, 2008), dragonflies (Pinkert *et al.*, 2017; Zeuss *et al.*, 2014), lady beetles (de Jong & Brakefield, 1998), Andean, European and Australian butterflies (Dufour *et al.*, 2018; Kang *et al.*, 2021; Munro *et al.*, 2019; Dalrymple *et al.*, 2008). Our results are surprising considering that small ectotherms depend more strongly on solar energy than endotherms (Gates, 1980). However, recent studies have shown that lightness/darkness patterns driven by humidity or photoprotection can override the effects of temperature (Law *et al.*, 2020; Delhey *et al.*, 2019). Our results could be explained by opposite gradients of humidity and temperature that create conflicting ecological challenges for some Christmas beetle species (Delhey *et al.*, 2019). Alternatively, Christmas beetles may have physiological or behavioural strategies for thermoregulation that reduce reliance on passive thermoregulation or even render it unnecessary (Addo-Bediako *et al.*, 2001; Rozen-Rechels *et al.*, 2019; Chown *et al.*, 2011). We emphasise that the species with a white underlay and extremely high NIR reflectivity and high NIR absorptivity (genus *Xylonychus* and *Paraschizognathus*, as well as *Anoplognathus prasinus*) (Ospina-Rozo *et al.*, 2022a) cluster in cooler environments (eastern coast of New South Wales, **Figure D-9**). Additional studies could compare the microhabitat of these species to identify the reasons behind this pattern. Despite these puzzling results, we cannot completely rule out the possibility that passive thermoregulation is relevant for some of the studied species instead of being a general selective pressure across the group.

None of our climate variables could explain variations in polarisation, and, contrary to expectations, polarisation was neither negatively nor positively correlated with visible reflectivity. Christmas beetles can use chiral nanostructures to reflect different amounts of light in the visible spectrum. The species *A. aureus*, *A. parvulus*, *A. roseus*, *A. laetus*, *A. opalinus* and *E. lamprimoides* all show high visible reflectivity and polarisation (**Figure D-8**) even though none of them can reflect both left and right

handed polarisation (unlike *Chrysina resplendens*, Finlayson *et al.*, 2017). Although a lot of work has been done mechanisms producing polarisation in golden scarabs (Goldstein, 2006; Libby *et al.*, 2014; Bagge *et al.*, 2020, amongst others), comparisons between these highly reflective species and their less reflective sister groups (such as the one in Parker *et al.*, 1998) are still needed to understand the level of modification required in the nanostructures to achieve different levels of reflectivity. For example, broadband golden reflectivity is achieved by incorporating uric acid to a chiral structure (Caveney, 1971), but the detailed composition of the chiral nanostructures in highly polarised beetles with darker narrow band reflectance and those with a faint golden sheen remains unexplored. Similarly, it could be interesting to study the intraspecific differences (observed here though not considered in our analysis) of some of the highly polarised species such as the red, green and blue morphs of *A. smaragdinus* or the red, pink and orange morphs of *A. laetus* (Jarvis, 1929). These species would be ideal to study the synergistic interactions between chemical substances and chiral structure, and the role of larval diet in the final appearances of the elytra. Meanwhile, the fact remains that chiral nanostructures can produce a wide range of appearances with different reflectivity in Christmas beetles, for which the evolutionary drivers are yet to be unravelled.

In our study, the accumulation of broadband pigments (likely melanin, Futahashi & Osanai-Futahashi, 2021; Cordero & Casadevall, 2020; Solano, 2014) reduced reflectivity in both visible and NIR, while the presence of structural colours (here measured as higher degree of polarisation) reduced only the NIR reflectivity (in agreement with Wang *et al.*, 2022a; Shawkey *et al.*, 2017). Three explanations are possible: 1) the selective pressures driving the evolution of polarisation and NIR reflectivity are opposite, 2) there is a specific trade-off in the underlying mechanisms or 3) the observed relationships are a by-product of the evolutionary history. In the first scenario, accumulation of melanin driven by selective pressures unrelated to overall reflectivity could cause generally less reflectance (across both visible and NIR), but its combination with chiral structures could result in more visible light (not NIR) reflected for communication, camouflage or other purposes. For the second scenario, proteins and molecules embedded around the chitin fibres (Lenau & Barfoed, 2008) could influence the indices of refraction in the chiral chitin fibres causing more NIR absorption. Large irregularities in the nanostructures could increase the number of surfaces to scatter NIR light, and every scattering event entails transmission of a proportion of the light into the material causing structural absorption/light trapping (Han *et al.*, 2013). The third scenario could arise in the absence of a strong selective

pressure for polarisation and NIR reflectivity. The highly iridescent NIR reflections of beetles *A. flavipennis* (former *A. brunnipennis* Seidel & Reid, 2021), *A. roseus*, *A. laetus*, *A. parvulus* and *A. aureus* (Ospina-Rozo *et al.*, 2022b), are remarkable exceptions to the general trend that deserve further investigation. Overall, the interplay between pigment-based and structural coloration allows flexibility in manipulating visible and NIR light, which may be a function of species-specific selective pressures. This possibility should be considered by cross-disciplinary studies combining the fields of evolutionary biology, analytical chemistry and photonics.

Are optical effects evolutionarily labile? Some studies have shown that structural colouration can be a key trait for diversification since subtle modifications in the spacing or shape of the nanostructures can create wildly different optical effects (White, 2018; Beltran *et al.*, 2021). Meanwhile, other studies indicate that structural colour is phylogenetically conserved due to the complex developmental and physiological mechanisms needed to produce nanostructures in the first place (Cooney *et al.*, 2019; Lloyd & Nadeau, 2021; Seago *et al.*, 2019). In Christmas beetles, reflectivity, absorptivity, and polarisation are phylogenetically constrained, but other optical effects such as iridescence, specularity and spectral location are not strongly correlated to the properties measured here and vary greatly even at the intraspecific level (Ospina-Rozo *et al.*, 2022b). These effects could be under different selective pressures and may be more labile. Notably, NIR reflectivity and polarisation - a hallmark of chiral nanostructures in beetles (Seago *et al.*, 2009) - showed higher phylogenetic signal than visible reflectivity, supporting the idea that there are evolutionary constraints on developing the first building blocks of structural colour, but once nanostructures are present in a group, some aspects of structural colour, such as angle-dependent properties, may be labile. To test this possibility an assembly of the phylogenetic relationships between Christmas beetles including more loci could provide a stronger basis for ancestral state reconstructions of optical properties in Christmas beetles and their underlying mechanisms. This, combined with biogeography (Allsopp, 1995) and microhabitat data could elucidate the evolutionary history of the striking and diverse optical effects produced by scarabs in the sub-family Rutelinae.

There are several reasons why correlations between optical properties and climate are complex and sometimes inconsistent, but a comparative approach is still a powerful tool to identify potential common selective pressures. Factors such as pleiotropy, developmental constraints, diet, larval habits and ecological challenges outside the activity period (e.g. resting) (Davis *et al.*, 2005; Karl *et al.*, 2008; Heidrich *et al.*, 2018;

Xing *et al.*, 2018; Costa *et al.*, 2008), signalling and camouflage (Cezário *et al.*, 2022; Kjærnsmo *et al.*, 2020; Kjærnsmo *et al.*, 2022), energy trade-offs affecting growth and reproduction, (Heidrich *et al.*, 2018; Mitov, 2017), multifunctionality (Schroeder *et al.*, 2018; He *et al.*, 2015; Rivera *et al.*, 2021; Su *et al.*, 2016), active microhabitat modification (Kaspari, 2019; Danks, 2002; Smolka *et al.*, 2012), physiological adaptations (Addo-Bediako *et al.*, 2001) and behaviour (Barton *et al.*, 2014; Tsai *et al.*, 2020; Nørgaard & Dacke, 2010; Pearson, 1988) can obscure trends at the macroecological scale (Goldenberg *et al.*, 2022). Since the diversity of optical effects in Christmas beetles may arise from species-specific combinations of these circumstances and multiple selective pressures, our results should be complemented with data on the fundamental biology of each species to understand the evolution of complex and diverse optical effects in beetles. Nevertheless, our macroecological analysis suggests that water regulation may be an important ecological challenge for Christmas beetles. It also highlights the complex interplay between structural and pigment-based colours in determining ecogeographical trends and enabled us to identify “special cases” that require further examination. Phylogenetic comparative studies provide a useful starting point for biophotonics studies searching to understand the causality between the structure of natural mechanisms of light manipulation and their function (Schroder-Turk, 2020).

5.6 | Data availability

In support of the Open Science Framework, find here a link and QR code to interactive web pages of my statistical analysis. This resource also provides access to the original data and permanent version tracking.

[Photonic Rebels code](#)



5.7 | CRediT Author Statement

Laura Ospina-Rozo: conceptualisation, investigation, formal analysis, data curation, visualisation and original draft. **Iliana Medina:** conceptualisation, software, validation, review. **Andrew Hugall:** Investigation, **Katrina J. Rankin:** Investigation and review, **Nicholas W. Roberts:** conceptualisation, writing- review and editing, **Andrew Mitchell:** resources and validation, **Chris A. M. Reid:** resources and validation, **Adnan Moussalli:** Investigation and resources, **Devi Stuart-fox:** conceptualisation, writing-review and editing, supervision.

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References

- COMSOL Multiphysics®. In: AB C, ed. v. 5.6. ed. Stockholm - Sweden.
- Addo-Bediako A, Chown SL, Gaston KJ. 2001. Revisiting water loss in insects: a large scale view. *Journal of Insect Physiology* **47**: 1377 - 1388.
- Adhikary SK, Ashish DK, Rudžionis Ž. 2021. Aerogel based thermal insulating cementitious composites: A review. *Energy and Buildings* **245**.
- Agez G, Bayon C, Mitov M. 2017. Multiwavelength micromirrors in the cuticle of scarab beetle *Chrysina gloriosa*. *Acta Biomater* **48**: 357-367.
- ALA. 2021. Records of the genus *Anoplognathus* - Australia. In: Australia AoL, ed.
- Alcock J. 1976. The behaviour of the seed-collecting larvae of a carabid beetle (Coleoptera). *Journal of Natural History* **10**: 367-375.
- Allsopp PG. 1995. Biogeography of the Australian Dynastinae, Rutelinae, Scarabaeinae, Melolonthini, Scitalini and Geotrupidae (Coleoptera: Scarabaeoidea). *Journal of Biogeography* **22**: 31-48.
- Alves VM, Hernandez MIM, Lobo JM. 2018. Elytra absorb ultraviolet radiation but transmit infrared radiation in neotropical *Canthon* species (Coleoptera, Scarabaeinae). *Photochemistry and Photobiology* **94**: 532-539.
- Amore V, Hernandez MIM, Carrascal LM, Lobo JM. 2017. Exoskeleton may influence the internal body temperatures of Neotropical dung beetles (Col. Scarabaeinae). *Peerj* **5**.
- Andersson S, Pryke SR, Ornborg J, Lawes MJ, Andersson M. 2002. Multiple Receivers, Multiple Ornaments, and a Trade-off between Agonistic and Epigamic Signaling in a Widowbird. *The American Naturalist* **160**.
- Andrew Richard P. 2000. 515 million years of structural colour. *Journal of Optics A: Pure and Applied Optics* **2**: R15.
- Arwin H, Magnusson R, Landin J, Järrendahl K. 2012. Chirality-induced polarization effects in the cuticle of scarab beetles: 100 years after Michelson. *Philosophical Magazine* **92**: 1583-1599.
- Azofeifa DE, Arguedas HJ, Vargas WE. 2012. Optical properties of chitin and chitosan biopolymers with application to structural color analysis. *Optical Materials* **35**: 175-183.
- Babarovic F, Puttick MN, Zaher M, Learmonth E, Gallimore EJ, Smithwick FM, Mayr G, Vinther J. 2019. Characterization of melanosomes involved in the production of non-iridescent structural feather colours and their detection in the fossil record. *Journal of the Royal Society Interface* **16**: 20180921.
- Bagge LE, Kenton AC, Lyons BA, Wehling MF, Goldstein DH. 2020. Mueller matrix characterizations of circularly polarized reflections from golden scarab beetles. *Applied Optics* **59**: F85-F93.
- Bagnara JT, Fernandez PJ, Fujii R. 2007. On the blue coloration of vertebrates. *Pigment Cell Research* **20**: 14-26.
- Bakken GS, Vanderbilt VC, Buttemer WA, Dawson WR. 1978. Avian Eggs: Thermoregulatory Value of Very High Near-Infrared Reflectance. *Science* **200**: 321-323.
- Barthlott W, Neinhuis C. 1997. Purity of the sacred lotus, or escape from contamination in biological surfaces. *Planta* **202**: 1-8.
- Bartholomew Ga, heinrich B. 1978. Endothermy in african dung beetles during flight, ball making, and ball rolling. *Journal of Experimental Biology* **73**: 65-83.
- Barton M, Porter W, Kearney M. 2014. Behavioural thermoregulation and the relative roles of convection and radiation in a basking butterfly. *Journal of Thermal Biology* **41**: 65-71.
- Beltran DF, Shultz AJ, Parra JL. 2021. Speciation rates are positively correlated with the rate of plumage color evolution in hummingbirds. *Evolution* **75**: 1665-1680.
- Benedek GB. 1971. Theory of transparency of the eye. *Applied Optics* **10**.

- Bermudez-Urena E, Kilchoer C, Lord NP, Steiner U, Wilts BD. 2020. Structural diversity with varying disorder enables the multicolored display in the longhorn beetle *Sulawesiella rafaellae*. *iScience* **23**: 101339.
- Berthier S. 2007. *Iridescences, The physical colours of insects*. Springer: New York, U.S.A.
- Bidau CJ, Martí DA. 2007. Clinal variation of body size in *Dichroplus pratensis* (Orthoptera: Acrididae): inversion of Bergmann's and Rensch's rules. *Annals of the Entomological Society of America* **100**: 850-860.
- Blaho M, Egri A, Hegedus R, Josvai J, Toth M, Kertesz K, Biro LP, Kriska G, Horvath G. 2012. No evidence for behavioral responses to circularly polarized light in four scarab beetle species with circularly polarizing exocuticle. *Physiology & Behavior* **105**: 1067-1075.
- Bolwig N. 1957. Experiments on the regulation of the body temperature of certain tenebrionid beetles. *Journal of the Entomological Society of Southern Africa* **20**: 454-458.
- Bonsignore CP, Bellamy C. 2007. Daily activity and flight behaviour of adults of *Capnodis tenebrionis* (Coleoptera: Buprestidae). *European Journal of Entomology* **104**: 425-431.
- Boyon C, Soldan V, Mitov M. 2021. Bioinspired, Cholesteric Liquid-Crystal Reflectors with Time-Controlled Coexisting Chiral and Achiral Structures. *ACS Applied Materials & Interfaces* **13**: 30118-30126.
- Brady P, Cummings M. 2010. Differential response to circularly polarized light by the jewel scarab beetle *Chrysina gloriosa*. *The American Naturalist* **175**: 614-620.
- Braun PV. 2011. Colour without colourants. *Nature* **472**.
- Brien MN, Enciso-Romero J, Lloyd VJ, Curran EV, Parnell AJ, Morochz C, Salazar PA, Rastas P, Zinn T, Nadeau NJ. 2022. The genetic basis of structural colour variation in mimetic *Heliconius* butterflies. *Philosophical Transactions of the Royal Society B: Biological Sciences* **377**: 20200505.
- Britton D. 2020. Christmas Beetle *Animal Facsheets*: Australian Museum.
- Burg SL, Parnell AJ. 2018. Self-assembling structural colour in nature. *Journal of Physics: Condensed Matter* **30**.
- Burg SL, Washington AL, Villanova J, Dennison AJC, McLoughlin D, Mykhaylyk OO, Vukusic P, Furnass W, Jones RAL, Parnell AJ, Fairclough JPA. 2020. X-ray nano-tomography of complete scales from the ultra-white beetles *Lepidiota stigma* and *Cyphochilus*. *Scientific Data* **7**: 163.
- Burresi M, Cortese L, Pattelli L, Kolle M, Vukusic P, Wiersma DS, Steiner U, Vignolini S. 2014. Bright-white beetle scales optimise multiple scattering of light. *Scientific Reports* **4**: 6075.
- Caglar SS, Karacaoglu C, Kuyucu AC, Saglam IK. 2013. Humidity and seasonality drives body size patterns in males of the bush cricket *Isophya rizeensis* Sevgili, 2003 (Orthoptera: Tettigoniidae: Phaneropterinae). *Insect Science* **21**: 213-226.
- Campos-Fernández C, Azofeifa DE, Hernández-Jiménez M, Ruiz-Ruiz A, Vargas WE. 2011. Visible light reflection spectra from cuticle layered materials. *Optical Materials Express* **1**.
- Careau V, Killen SS, Metcalfe NB. 2015. Adding fuel to the "fire of life": energy budgets across levels of variation in ectotherms and endotherms. In: Martin LB, Ghalambor CK and Woods HA, eds. *Integrative Organismal Biology*: John Wiley & Sons Inc (US).
- Carlson DC. 1980. Taxonomic Revision of *Lichnanthe Burmeister* (Coleoptera: Scarabaeidae). *The Coleopterists Society* **34**.
- Carne PB. 1957. A revision of the ruteline genus *Anoplognathus* Leach (Coleoptera Scarabaeidae). *Australian Journal of Zoology* **6**: 162-240.
- Carne PB. 1958. A review of the Australian rutelinae (Coleoptera: Scarabaeidae). *Australian Journal of Zoology* **6**: 162-240.

- Carne PB. 1974. A review of the olivaceous species-group of the genus *Paraschizognathus* Ohaus, and description of three new species (coleoptera : scarabaeidae). *Journal of the Australian Entomological Society* 13.
- Carney RM, Vinther J, Shawkey MD, D'alba L, Ackermann J. 2012. New evidence on the colour and nature of the isolated Archaeopteryx feather. *Nature communications* 3: 1-6.
- Caro T, Stoddard MC, Stuart-Fox D. 2017. Animal coloration research: why it matters. *Philosophical Transactions of the Royal Society B: Biological Sciences* 372.
- Carrascal LM, Ruiz YJ, Lobo JM. 2017. Beetle exoskeleton may facilitate body heat acting differentially across the electromagnetic spectrum. *Physiological and Biochemical Zoology* 90: 338-347.
- Casey TM. 1992. Biophysical ecology and heat exchange in insects. *American Zoologist* 32: 225-237.
- Cassis GW, T. A. 1992. *Zoological catalogue of Australia Volume 9 - Coleoptera: Scarabaeoidea*. Australian Govt. Pub. Service: Canberra.
- Caveney S. 1971. Cuticle reflectivity and optical activity in scarab beetles: the role of uric acid. *Proceedings of the Royal Society B: Biological Sciences* 178: 205-225.
- Cezário RR, Gorb SN, Guillermo-Ferreira R. 2022. Camouflage by counter-brightness: the blue wings of *Morpho* dragonflies *Zenithoptera lanei* (Anisoptera: Libellulidae) match the water background. *Journal of Zoology* 317: 92-100.
- Chen J, Hao N, Pan L, Hu L, Du S, Fu Y. 2020a. Characteristics of compressive mechanical properties and strengthening mechanism of 3D-printed grid beetle elytron plates. *Journal of Materials Science* 55: 8541-8552.
- Chen J, Zhang X, Okabe Y, Xie J, Xu M. 2018. Beetle elytron plate and the synergistic mechanism of a trabecular-honeycomb core structure. *Science China Technological Sciences* 62: 87-93.
- Chen M, Pang D, Mandal J, Chen X, Yan H, He Y, Yu N, Yang Y. 2021a. Designing Mesoporous Photonic Structures for High-Performance Passive Daytime Radiative Cooling. *Nano Letters* 21: 1412-1418.
- Chen Y, Mandal J, Li W, Smith-Washington A, Tsai C-C, Huang W, Shrestha S, Yu N, Han RPS, Cao A, Yang Y. 2020b. Colored and paintable bilayer coatings with high solar-infrared reflectance for efficient cooling. *Science Advances* 6.
- Chen Z, Zhang Z, Wang Y, Xu D, Zhao Y. 2021b. Butterfly inspired functional materials. *Materials Science and Engineering: R: Reports* 144.
- Cheng W, Xing S, Chen YI, Lin R, Bonebrake TC, Nakamura A. 2018. Dark butterflies camouflaged from predation in dark tropical forest understories. *Ecological Entomology* 43: 304-309.
- Chiou TH, Kleinlogel S, Cronin T, Caldwell R, Loeffler B, Siddiqi A, Goldizen A, Marshall J. 2008. Circular polarization vision in a stomatopod crustacean. *Current Biology* 18: 429-434.
- Choi SH, Byun KM. 2019. Naturally occurring order-disorder duality in photonic structures of the *Haliotis fulgens* abalone shell. *Optical Materials Express* 9.
- Chown SL, Gaston KJ. 2010. Body size variation in insects: a macroecological perspective. *Biological Reviews* 85: 139-169.
- Chown SL, Sorensen JG, Terblanche JS. 2011. Water loss in insects: an environmental change perspective. *Journal Insect Physiology* 57: 1070-1084.
- Clusella-Trullas S, Blackburn TM, Chown SL. 2011. Climatic predictors of temperature performance curve parameters in ectotherms imply complex responses to climate change. *The American Naturalist* 177: 738-751.
- Clusella-Trullas S, Nielsen M. 2020. The evolution of insect body coloration under changing climates. *Current opinion in insect science* 41: 25-32.
- Clusella-Trullas S, Terblanche JS, Blackburn TM, Chown SL. 2008. Testing the thermal melanism hypothesis: a macrophysiological approach. *Functional Ecology* 22: 232-238.

- Clusella Trullas S, van Wyk JH, Spotila JR. 2007. Thermal melanism in ectotherms. *Journal of Thermal Biology* 32: 235-245.
- Cook CQ, Amir A. 2016. Theory of chirped photonic crystals in biological broadband reflectors. *Optica* 3: 1436-1439.
- Cooney CR, Varley ZK, Nouri LO, Moody CJA, Jardine MD, Thomas GH. 2019. Sexual selection predicts the rate and direction of colour divergence in a large avian radiation. *Nature communications* 10: 1773.
- Cordero RJB, Casadevall A. 2020. Melanin. *Current Biology* 30: R142-R143.
- Costa GC, Vitt LJ, Pianka ER, Mesquita DO, Colli GR. 2008. Optimal foraging constrains macroecological patterns: body size and dietary niche breadth in lizards. *Global Ecology and Biogeography* 17: 670-677.
- Crowson RA. 2013. *The biology of the Coleoptera*. Academic press.
- CSIRO Bureau of Meteorology. 2014. State of the Climate: Commonwealth of Australia.
- CSIRO. Division of Entomology, Naumann ID. 1991. Insects of Australia. Second edition. ed. 1 online resource (PDF).
- Cuesta E, Lobo JM. 2019. Visible and near-infrared radiation may be transmitted or absorbed differently by beetle elytra according to habitat preference. *Peerj* 7.
- Cuthill IC, Allen WL, Arbuckle K, Caspers B, Chaplin G, Hauber ME, Hill GE, Jablonski NG, Jiggins CD, Kelber A, Mappes J, Marshall J, Merrill R, Osorio D, Prum R, Roberts NW, Roulin A, Rowland HM, Sherratt TN, Skelhorn J, Speed MP, Stevens M, Stoddard MC, Stuart-Fox D, Talas L, Tibbetts E, Caro T. 2017. The Biology of Colour. *Science* 357: 6350.
- Cuthill IC, Bennett ATD, Partridge JC, Maier EJ. 1999. Plumage Reflectance and the Objective Assessment of Avian Sexual Dichromatism. *The American Naturalist* 160.
- Dacke M, Nordström P, Scholtz CH. 2003. Twilight orientation to polarised light in the crepuscular dung beetle *Scarabaeus zambesianus*. *Journal of Experimental Biology* 206: 1535-1543.
- Dacke M, Nordstrom P, Scholtz CH, Warrant EJ. 2002. A specialized dorsal rim area for polarized light detection in the compound eye of the scarab beetle *Pachysoma striatum*. *Journal of Comparative Physiology A* 188: 211-216.
- Dalrymple RI, flores-moreno H, Kemp DJ, White Te, Laffan Sw, Hemmings FA, Hitchcock Td, Moles AT. 2008. Abiotic and biotic predictors of macroecological patterns in bird and butterfly coloration. *Ecological Monographs* 88: 204-224.
- Danks HV. 2002. Modification of adverse conditions by insects. *Oikos* 99.
- Davis AK, Farrey BD, Altizer S. 2005. Variation in thermally induced melanism in monarch butterflies (Lepidoptera: Nymphalidae) from three North American populations. *Journal of Thermal Biology* 30: 410-421.
- Dawson TJ, Webster KN, Maloney SK. 2014. The fur of mammals in exposed environments; do crypsis and thermal needs necessarily conflict? The polar bear and marsupial koala compared. *Journal of Comparative Physiology B* 184: 273-284.
- de Jong PW, Brakefield PM. 1998. Climate and change in clines for melanism in the two-spot ladybird, *Adalia bipunctata* (Coleoptera: Coccinellidae). *Proceedings of the Royal Society of London. Series B: Biological Sciences* 265: 39-43.
- Deerinck TJ, Bushong EA, Lev-Ram V, Shu X, Tsien RY, Ellisman MH. 2010. Enhancing Serial Block-Face Scanning Electron Microscopy to Enable High Resolution 3-D Nanohistology of Cells and Tissues. *Microscopy and Microanalysis* 16: 1138-1139.
- Delhey K. 2018. Darker where cold and wet: Australian birds follow their own version of Gloger's rule. *Ecography* 41: 673-683.
- Delhey K. 2019. A review of Gloger's rule, an ecogeographical rule of colour: definitions, interpretations and evidence. *Biological Reviews* 94: 1294-1316.

- Delhey K, Dale J, Valcu M, Kempenaers B. 2019. Reconciling ecogeographical rules: rainfall and temperature predict global colour variation in the largest bird radiation. *Ecology Letters* 22: 726-736.
- Denton E. 1971. Reflectors in fishes. *Scientific American* 224: 64-75.
- Deparis O, Rassart M, Vandenbem C, Welch V, Vigneron JP, Lucas S. 2008. Structurally tuned iridescent surfaces inspired by nature. *New Journal of Physics* 10.
- Dobzhansky T. 1933. Geographical Variation in Lady-Beetles. *The American Naturalist* 67: 97-126.
- Doucet SM, Hill GE. 2009. Do museum specimens accurately represent wild birds? A case study of carotenoid, melanin, and structural colours in long-tailed manakins *Chiroxiphia linearis*. *Journal of Avian Biology* 40: 146-156.
- Doucet SM, Meadows MG. 2009. Iridescence: a functional perspective. *Journal of the Royal Society Interface* 6 Suppl 2: S115-132.
- Draney ML. 1993. The subelytral cavity of desert tenebrionids. *Florida Entomologist*: 539-549.
- Dufour PC, Willmott KR, Padrón PS, Xing S, Bonebrake TC, Scheffers BR. 2018. Divergent melanism strategies in Andean butterfly communities structure diversity patterns and climate responses. *Journal of Biogeography* 45: 2471-2482.
- Duncan FD. 2002. The role of the subelytral cavity in water loss in the flightless dung beetle, *Circellium bacchus* (Coleoptera: Scarabaeinae). *European Journal of Entomology* 99: 253-258.
- Duncan FD. 2021. Respiratory Strategies in Relation to Ecology and Behaviour in Three Diurnal Namib Desert Tenebrionid Beetles. *Insects* 12.
- Dzialowski EM. 2005. Use of operative temperature and standard operative temperature models in thermal biology. *Journal of Thermal Biology* 30: 317-334.
- Echeverri SA, Miller AE, Chen J, McQueen EW, Plakke M, Spicer M, Hoke KL, Stoddard MC, Morehouse NI. 2021. How Signaling Geometry Shapes the Efficacy and Evolution of Animal Communication Systems. *Integrative and Comparative Biology* 61: 787-813.
- Edwards PB, Wanjura WJ, Brown WV. 1993. Selective herbivory by Christmas beetles in response to intraspecific variation in *Eucalyptus* terpenoids. *Oecologia* 95.
- Eliason CM, Maia R, Parra JL, Shawkey MD. 2020. Signal evolution and morphological complexity in hummingbirds (Aves: Trochilidae). *Evolution* 74: 447-458.
- Eliason CM, Shawkey MD. 2011. Decreased hydrophobicity of iridescent feathers: a potential cost of shiny plumage. *Journal of Experimental Biology* 214: 2157-2163.
- Eliason CM, Shawkey MD, Clarke JA. 2016. Evolutionary shifts in the melanin-based color system of birds. *Evolution* 70: 445-455.
- Endler JA. 1990. On the measurement and classification of colour in studies of animal colour patterns. *Biological Journal of the Linnean Society* 41: 315-352.
- Endler JA. 1993. The color of light in forests and its implications. *Ecological Monographs* 63: 1-27.
- Endler JA, Mappes J. 2017. The current and future state of animal coloration research. *Philosophical Transactions of the Royal Society B: Biological Sciences* 372.
- Endler JA, Mielke Jr PW. 2005. Comparing entire colour patterns as birds see them. *Biological Journal of the Linnean Society* 86: 405 - 431.
- Evison SEF, Gallagher JD, Thompson JJW, Siva-Jothy MT, Armitage SAO. 2017. Cuticular colour reflects underlying architecture and is affected by a limiting resource. *Journal of Insect Physiology* 98: 7-13.
- Fedrigo O, Wray GA. 2010. Developmental Evolution: How Beetles Evolved Their Shields. *Current Biology* 20.

- Feller KD, Jordan TM, Wilby D, Roberts NW. 2017. Selection of the intrinsic polarization properties of animal optical materials creates enhanced structural reflectivity and camouflage. *Philosophical Transactions of the Royal Society B: Biological Sciences* 372.
- Finlayson ED, McDonald LT, Vukusic P. 2017. Optically ambidextrous circularly polarized reflection from the chiral cuticle of the scarab beetle *Chrysina resplendens*. *Journal of the Royal Society Interface* 14.
- Forsman A, Ringblom K, Civantos E, Ahnesjö J. 2002. Coevolution of color pattern and thermoregulatory behavior in polymorphic pygmy grasshoppers *Tetrix undulata*. *Evolution* 56: 349-360.
- Fox DL. 1976. *Animal biochromes and structural colours: physical, chemical, distributional & physiological features of coloured bodies in the animal world*. Univ of California Press.
- Fox HM, Vevers G. 1960. *Nature of animal colours*. Sidgwick and Jackson.
- Franklin AM, Ospina-Rozo L. 2021. Gloss. *Current Biology* 31: R172-R173.
- Franklin AM, Rankin KJ, Ospina-Rozo L, Medina I, Garcia JE, Ng L, Dong C, Wang LY, Aulsebrook AE, Stuart-Fox D. 2021. Cracks in the mirror hypothesis: High specularity does not reduce detection or predation risk. *Functional Ecology*.
- Fu J, Yoon BJ, Park JO, Srinivasarao M. 2017. Imaging optical scattering of butterfly wing scales with a microscope. *Interface Focus* 7: 20170016.
- Futahashi R, Osanai-Futahashi M. 2021. Pigments in Insects *Pigments, Pigment Cells and Pigment Patterns*. 3-43.
- Galván I, Rodríguez-Martínez S, Carrascal LM, Portugal S. 2018. Dark pigmentation limits thermal niche position in birds. *Functional Ecology* 32: 1531-1540.
- Galván I, Sanz JJ. 2010. Measuring plumage colour using different spectrophotometric techniques: a word of caution. *Ornis Fennica* 87: 87-89.
- Gans C, Baic D. 1977. Regional specialization of reptilian scale surfaces: relation of texture and biologic role. *Science* 195: 1348-1350.
- Garcia JE, Shrestha M, Ospina-Rozo L, Dekiwadia C, Field MR, Ma JS, Tran N, Dyer AG, Fox K, Greentree AD. 2020. Iridescence and hydrophobicity have no clear delineation that explains flower petal micro-surface. *Scientific Reports* 10: 10685.
- Gates DM. 1980. Energy budgets of animals *Biophysical Ecology*. New York, NY: Springer New York. 382-462.
- Gausman HW, Allen WA, Cardenas R. 1969. Reflectance of cotton leaves and their structure. *Remote Sensing of Environment* 1.
- Ghiradella H. 1999. Shining armor: structural colors in insects. *Optics and Photonics News* 10: 46-48.
- Giraldo MA, Parra JL, Stavenga DG. 2018. Iridescent colouration of male Anna's hummingbird (*Calypte anna*) caused by multilayered barbules. *Journal of Comparative Physiology A* 204: 965-975.
- Girard MB, Endler JA. 2014. Peacock spiders. *Current Biology* 24: R588-590.
- Gokan N. 1982. The compound eye of the soybean beetle, *Anomala rufocuprea* Motschulsky (Coleoptera: Scarabaeidae). *Applied Entomology and Zoology* 17: 227-237.
- Goldenberg J, Bisschop K, D'Alba L, Shawkey MD. 2022. The link between body size, colouration and thermoregulation and their integration into ecogeographical rules: a critical appraisal in light of climate change. *Oikos* 2022.
- Goldstein DH. 2006. Polarization properties of Scarabaeidae. *Applied Optics* 45: 7944-7950.
- Gomez D, Pinna C, Pairraire J, Arias Mn, Barbut Jr, Pomerantz A, Daney de Marcillac W, Berthier S, Patel N, Andraud C, Elias M. 2021. Wing transparency in butterflies and moths: structural diversity, optical properties, and ecological relevance. *Ecological Monographs* 91.

- Gonome H, Nakamura M, Okajima J, Maruyama S. 2018. Artificial chameleon skin that controls spectral radiation: Development of Chameleon Cool Coating (C(3)). *Scientific Reports* 8: 1196.
- Gower DJ. 2003. Scale microornamentation of uropeltid snakes. *Journal of Morphology* 258: 249-268.
- Grether GF, Kolluru GR, Nersissian K. 2004. Individual colour patches as multicomponent signals. *Biological Reviews* 79: 583-610.
- Grimaldi D, Engel MS, Engel MS, Engel MS. 2005. *Evolution of the Insects*. Cambridge University Press.
- Gronquist M, Schroeder FC, Ghiradella H, Hill D, McCoy EM, Meinwald J, Eisner T. 2006. Shunning the night to elude the hunter: diurnal fireflies and the “femmes fatales”. *Chemoecology* 16: 39-43.
- Gruson H, Andraud C, Daney de Marcillac W, Berthier S, Elias M, Gomez D. 2019a. Quantitative characterization of iridescent colours in biological studies: a novel method using optical theory. *Interface Focus* 9: 20180049.
- Gruson H, Elias M, Andraud C, Djediat C, Berthier S, Doutrelant C, Gomez D. 2019b. Hummingbird iridescence: an unsuspected structural diversity influences colouration at multiple scales. *Preprint at bioRxiv*.
- Gunderson SL, Gunnison KE, Sawvel JW. 1991. Hierarchical Structure of a natural composite: Insect cuticle. *MRS Online Proceedings Library (OPL)* 255.
- Gunter NL, Weir TA, Slipinski A, Bocak L, Cameron SL. 2016. If dung beetles (Scarabaeidae: Scarabaeinae) arose in association with dinosaurs, did they also suffer a mass co-extinction at the K-Pg boundary? *PLoS One* 11: e0153570.
- Gur D, Palmer BA, Weiner S, Addadi L. 2017. Light Manipulation by Guanine Crystals in Organisms: Biogenic Scatterers, Mirrors, Multilayer Reflectors and Photonic Crystals. *Advanced Functional Materials* 27.
- Hadley NF, Savill A, Schultz TD. 1992. Coloration and its thermal consequences in the New Zealand tiger beetle *Neocicindela perhispidia*. *Journal of Thermal Biology* 17: 55 - 61.
- Hadley NF, Schultz TD, Savill A. 1988. Spectral reflectances of three tiger beetle subspecies (*Neocicindela perhispidia*): Correlations with their habitat substrate. *New Zealand Journal of Zoology* 15: 343-346.
- Halfpeter G. 1991. Historical and ecological factors determining the geographical distribution of beetles (Coleoptera: Scarabaeidae: Scarabaeinae). *Biogeographia - The Journal of Integrative Biogeography* 15.
- Hallam BT, Hiorns AG, Vukusic P. 2009. Developing optical efficiency through optimized coating structure: biomimetic inspiration from white beetles. *Applied Optics* 48: 3243-3249.
- Halsey LG. 2019. The reign of the p-value is over: what alternative analyses could we employ to fill the power vacuum? *Biology Letters* 15: 20190174.
- Han Z-w, Wu L-y, Qiu Z-m, Guan H-y, Ren L-q. 2008. Structural colour in butterfly *Apatura ilia* scales and the microstructure simulation of photonic crystal. *Journal of Bionic Engineering* 5: 14-19.
- Han Z, Niu S, Zhang L, Liu Z, Ren L. 2013. Light trapping effect in wing scales of butterfly *Papilio peranthus* and its simulations. *Journal of Bionic Engineering* 10: 162-169.
- Hangay G, Zborowski P. 2010. *A Guide to the Beetles of Australia*. CSIRO Publishing.
- Hapke B. 2017. Reflectance Methods and Applications *Encyclopedia of Spectroscopy and Spectrometry*. 931-935.
- Harris R, McQuillan P, Hughes L. 2012. Patterns in body size and melanism along a latitudinal cline in the wingless grasshopper, *Phaulacridium vittatum*. *Journal of Biogeography* 39: 1450-1461.
- Harrison JF. 2009. Tracheal system *Encyclopedia of insects*: Elsevier. 1011-1015.
- He C, Zu Q, Chen J, Noori MN. 2015. A review of the mechanical properties of beetle elytra and development of the biomimetic honeycomb plates. *Journal of Sandwich Structures & Materials* 17: 399-416.

- Heidrich L, Friess N, Fiedler K, Brändle M, Hausmann A, Brandl R, Zeuss D. 2018. The dark side of Lepidoptera: Colour lightness of geometrid moths decreases with increasing latitude. *Global Ecology and Biogeography* 27: 407-416.
- Heinrich B. 1993. Beetles large and small *The Hot-Blooded Insects, Strategies and Mechanisms of Thermoregulation*. 1 ed: Springer, Berlin, Heidelberg and Harvard University Press.
- Hernández-Jiménez M, Azofeifa DE, Libby E, Barboza-Aguilar C, Solís Á, Arce-Marengo L, García-Aguilar I, Hernández A, Vargas WE. 2014. Qualitative correlation between structural chirality through the cuticle of *Chrysina aurigans* scarabs and left-handed circular polarization of the reflected light. *Optical Materials Express* 4.
- Hill GE, Hill GE, McGraw KJ, Kevin J. 2006. *Bird coloration, volume 1: mechanisms and measurements*. Harvard University Press.
- Hogan BG, Stoddard MC. 2018. Synchronization of speed, sound and iridescent color in a hummingbird aerial courtship dive. *Nature communications* 9: 1-8.
- Horváth G, Blahó M, Egri Á, Hegedüs R, Szél G. 2014. Circular Polarization Vision of Scarab Beetles. In: Horváth G, ed. *Polarized Light and Polarization Vision in Animal Sciences. Springer Series in Vision Research*. Berlin, Heidelberg: Springer.
- Hurtado P, Prieto M, Martinez-Vilalta J, Giordani P, Aragon G, Lopez-Angulo J, Kosuthova A, Merinero S, Diaz-Pena EM, Rosas T, Benesperi R, Bianchi E, Grube M, Mayrhofer H, Nascimbene J, Wedin M, Westberg M, Martinez I. 2020. Disentangling functional trait variation and covariation in epiphytic lichens along a continent-wide latitudinal gradient. *Proceedings of the Royal Society B* 287: 20192862.
- Huxley J. 1975. The basis of structural colour variation in two species of *Papilio*. *Journal of Entomology Series A, General Entomology* 50: 9-22.
- Imafuku M, Ogihara N. 2016. Wing Scale Orientation Alters Reflection Directions in the Green Hairstreak *Chrysozephyrus smaragdinus* (Lycaenidae; Lepidoptera). *Zoological Science* 33: 616-622.
- Iskandar J-P, Eliason CM, Astrop T, Igic B, Maia R, Shawkey MD. 2016. Morphological basis of glossy red plumage colours. *Biological Journal of the Linnean Society* 119: 477-487.
- Iwamoto M, Ched J, Kurashiki K, Ni Q-Q. 2002. Chitin Fibre And Its Laminated Structure Of The Fore-wing Of Beetle. *High Performance Structures and Composites* 59.
- Järrendahl K, Arwin H. 2018. Polarizing Natural Nanostructures *Ellipsometry of Functional Organic Surfaces and Films*. 247-268.
- Jarvis E. 1929. Some notes on the economy of cockchafer beetles. *Bureau of sugar experiment stations* 20.
- Johansen VE, Onelli OD, Steiner LM, Vignolini S. 2017. Photonics in Nature: From Order to Disorder. In: Gorb S and Gorb E, eds. *Functional Surfaces in Biology III. Biologically-Inspired Systems*.
- John HA. 1988. Mimetic Tiger Beetles and the Puzzle of Cicindelid Coloration (Coleoptera: Cicindelidae). *The Coleopterists Bulletin* 42: 28-33.
- Johns CV, Stone C, Hughes L. 2004. Feeding preferences of the Christmas beetle *Anoplognathus chloropyrus* (Coleoptera: Scarabaeidae) and four paropsine species (Coleoptera: Chrysomelidae) on selected *Eucalyptus grandis* clonal foliage. *Australian Forestry* 67.
- Johnsen S. 2012. *The Optics of Life: A Biologist's Guide to Light in Nature*. Princeton University Press.
- Johnsen S. 2016. How to measure color using spectrometers and calibrated photographs. *Journal of Experimental Biology* 219: 772-778.
- Jordan TM, Partridge JC, Roberts NW. 2014. Disordered animal multilayer reflectors and the localization of light. *Journal of the Royal Society Interface* 11: 20140948.

- Kang C, Im S, Lee WY, Choi Y, Stuart-Fox D, Huertas B. 2021. Climate predicts both visible and near-infrared reflectance in butterflies. *Ecology Letters* **24**: 1869-1879.
- Karl I, A. Janowitz S, Fischer K. 2008. Altitudinal life-history variation and thermal adaptation in the copper butterfly *Lycaena tityrus*. *Oikos* **0**: 080227085440234-080227085440230.
- Kaspri M. 2019. In a globally warming world, insects act locally to manipulate their own microclimate. *Proceedings of the National Academy of Sciences* **116**: 5220-5222.
- Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP, Webb CO. 2010. Picante: {R} tools for integrating phylogenies and ecology. *Bioinformatics* **26**: 1463-1464.
- Kemp DJ. 2002. Shedding new light on nature's brightest signals. *Trends in Ecology & Evolution* **17**.
- Kemp DJ, Herberstein ME, Fleishman LJ, Endler JA, Bennett AT, Dyer AG, Hart NS, Marshall J, Whiting MJ. 2015. An integrative framework for the appraisal of coloration in nature. *The American Naturalist* **185**: 705-724.
- Kemp DJ, Rutowski RL. 2007. Condition dependence, quantitative genetics, and the potential signal content of iridescent ultraviolet butterfly coloration. *Evolution* **61**: 168-183.
- Kemp DJ, Rutowski RL, Mendoza M. 2005. Colour pattern evolution in butterflies: A phylogenetic analysis of structural ultraviolet and melanic markings in North American sulphurs. *Evolutionary Ecology Research* **7**: 133-141.
- Kinoshita S, Yoshioka S. 2005. Structural colors in nature: the role of regularity and irregularity in the structure. *Chemphyschem* **6**: 1442-1459.
- Kinoshita S, Yoshioka S, Kawagoe K. 2002. Mechanisms of structural colour in the Morpho butterfly: cooperation of regularity and irregularity in an iridescent scale. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **269**: 1417-1421.
- Kinoshita S, Yoshioka S, Miyazaki J. 2008. Physics of structural colors. *Reports on Progress in Physics* **71**: 076401.
- Kjernsmo K, Hall JR, Doyle C, Khuzayim N, Cuthill IC, Scott-Samuel NE, Whitney HM. 2018. Iridescence impairs object recognition in bumblebees. *Scientific Reports* **8**: 8095.
- Kjernsmo K, Lim AM, Middleton R, Hall JR, Costello LM, Whitney HM, Scott-Samuel NE, Cuthill IC. 2022. Beetle iridescence induces an avoidance response in naïve avian predators. *Animal Behaviour* **188**: 45-50.
- Kjernsmo K, Whitney HM, Scott-Samuel NE, Hall JR, Knowles H, Talas L, Cuthill IC. 2020. Iridescence as Camouflage. *Current Biology* **30**: 551-555 e553.
- Knisley CB, Schultz TD. 1997. *The biology of tiger beetles and a guide to the species of the South Atlantic States*. Virginia Museum of Natural.
- Kozlov MV, Oudendijk Z, Forsman A, Lanta V, Barclay MVL, Gusarov VI, Gustafsson B, Huang ZZ, Kruglova OY, Marusik YM, Mikhailov YE, Mutanen M, Schneider A, Sekerka L, Sergeev ME, Zverev V, Zvereva EL. 2022. Climate shapes the spatiotemporal variation in color morph diversity and composition across the distribution range of *Chrysomela lapponica* leaf beetle. *Insect Science* **29**: 942-955.
- Kremer JR, Mastrorade DN, McIntosh R. 1996. Computer Visualization of Three-Dimensional Image Data Using IMOD. *Journal of Structural Biology* **116**: 71-76.
- Kundanati L, Signetti S, Gupta HS, Menegon M, Pugno NM. 2018. Multilayer stag beetle elytra perform better under external loading via non-symmetric bending properties. *Journal of the Royal Society Interface* **15**.
- Labhart T, Meyer EP, Schenker L. 1992. Specialized ommatidia for polarization vision in the compound eye of cockchafers, *Melolontha melolontha* (Coleoptera, Scarabaeidae). *Cell Tissue Research* **268**: 4190429.

- Lafaita J, Andraud C, Berthier S, Boulenguez J, Callet P, Dumazet S, Rassart M, Vigner J-P. 2010. Modeling the vivid white color of the beetle *Calothyrza margaritifera*. *Materials Science and Engineering B* 169.
- Law SJ, Bishop TR, Eggleton P, Griffiths H, Ashton L, Parr C. 2020. Darker ants dominate the canopy: Testing macroecological hypotheses for patterns in colour along a microclimatic gradient. *Journal of Animal Ecology* 89: 347-359.
- Lawrence JF, Britton EB. 1994. *Australian beetles*. Melbourne University Press.
- Lawrence JF, Newton AF. 1982. Evolution and Classification of Beetles. *Annual Review of Ecology and Systematics* 13: 261 - 290.
- Lee SH, Han SM, Han SE. 2020. Anisotropic diffusion in *Cyphochilus* white beetle scales. *APL Photonics* 5.
- Leertouwer HL, Wilts BD, Stavenga DG. 2011. Refractive index and dispersion of butterfly chitin and bird keratin measured by polarizing interference microscopy. *Optics Express* 19: 24061-24066.
- Lenau T, Barfoed M. 2008. Colours and Metallic Sheen in Beetle Shells – A Biomimetic Search for Material Structuring Principles Causing Light Interference. *Advanced Engineering Materials* 10: 299-314.
- Libby E, Azofeifa DE, Hernández-Jiménez M, Barboza-Aguilar C, Solís A, García-Aguilar I, Arce-Marengo L, Hernández A, Vargas WE. 2014. Light reflection by the cuticle of *C. aurigans* scarabs: a biological broadband reflector of left handed circularly polarized light. *Journal of Optics* 16.
- Linz DM, Hu AW, Sitvarin MI, Tomoyasu Y. 2016. Functional value of elytra under various stresses in the red flour beetle, *Tribolium castaneum*. *Scientific Reports* 6: 34813.
- Lippens S, Kremer A, Borghgraef P, Guerin CJ. 2019. Serial block face-scanning electron microscopy for volume electron microscopy. *Methods in cell biology* 152: 69-85.
- Lloyd VJ, Nadeau NJ. 2021. The evolution of structural colour in butterflies. *Current opinion in genetics & development* 69: 28-34.
- Lomakin J, Huber PA, Eichler C, Arakane Y, Kramer KJ, Beeman RW, Kanost MR, Gehrke SH. 2011. Mechanical Properties of the Beetle Elytron, a Biological Composite Material. *Biomacromolecules* 12: 321-335.
- Luke CA. 1994. Evolution of color change in desert lizards. *Herpetology of North American Deserts: Proceedings of a Symposium* (PR Brown and JW Wright, eds.). Southwestern Herpetologists Society, USA, 143-147.
- Luke SM, Hallam BT, Vukusic P. 2010. Structural optimization for broadband scattering in several ultra-thin white beetle scales. *Applied Optics* 49: 4246-4254.
- Magkiriadou S, Park JG, Kim YS, Manoharan VN. 2014. Absence of red structural color in photonic glasses, bird feathers, and certain beetles. *Physical Review E* 90: 062302.
- Maia R, D'Alba L, Shawkey MD. 2011. What makes a feather shine? A nanostructural basis for glossy black colours in feathers. *Proceedings of the Royal Society B: Biological Sciences* 278: 1973-1980.
- Maia R, Gruson H, Endler JA, White TE, O'Hara RB. 2019. pavo 2: New tools for the spectral and spatial analysis of colour in R. *Methods in Ecology and Evolution* 10: 1097-1107.
- Maia R, Rubenstein DR, Shawkey MD. 2016. Selection, constraint, and the evolution of coloration in African starlings. *Evolution* 70: 1064-1079.
- Mänd T, Tammaru T, Mappes J. 2007. Size dependent predation risk in cryptic and conspicuous insects. *Evolutionary Ecology* 21.
- Mandal J, Fu Y, Overvig A, Jia M, Sun K, Shi N, Zhou H, Xiao X, Yu N, Yang Y. 2018. Hierarchically porous polymer coatings for highly efficient passive daytime radiative cooling. *Science* 362: 315-319.
- Mandal J, Yang Y, Yu N, Raman AP. 2020. Paints as a Scalable and Effective Radiative Cooling Technology for Buildings. *Joule* 4: 1350-1356.

- Marshall NJ, Powell SB, Cronin TW, Caldwell RL, Johnsen S, Gruev V, Chiou TS, Roberts NW, How MJ. 2019. Polarisation signals: a new currency for communication. *Journal of Experimental Biology* **222**.
- Mathger LM, Denton EJ, Marshall NJ, Hanlon RT. 2009. Mechanisms and behavioural functions of structural coloration in cephalopods. *Journal of the Royal Society Interface* **6 Suppl 2**: S149-163.
- Matsuki M, Foley WJ, Floyd RB. 2011. Role of volatile and non-volatile plant secondary metabolites in host tree selection by Christmas beetles. *Journal Chemical Ecology* **37**: 286-300.
- McCoy DE, Feo T, Harvey TA, Prum RO. 2018. Structural absorption by barbule microstructures of super black bird of paradise feathers. *Nature communications* **9**: 1.
- McCoy DE, Prum RO. 2019. Convergent evolution of super black plumage near bright color in 15 bird families. *Journal of Experimental Biology* **222**.
- McDonald LT, Finlayson ED, Wilts BD, Vukusic P. 2017. Circularly polarized reflection from the scarab beetle *Chalcothea smaragdina*: light scattering by a dual photonic structure. *Interface Focus* **7**: 20160129.
- McGraw KJ. 2004. Multiple UV reflectance peaks in the iridescent neck feathers of pigeons. *The Science of Nature* **91**: 125-129.
- McNamara ME, Briggs DE, Orr PJ, Noh H, Cao H. 2012. The original colours of fossil beetles. *Proceedings of the Royal Society B: Biological Sciences* **279**: 1114-1121.
- McNamara ME, Orr P. 2013. The taphonomy of colour in fossil insects and feathers. *Palaeontology* **56**: 557-575.
- Meadows MG, Butler MW, Morehouse NI, Taylor LA, Toomey MB, McGraw KJ, Rutowski RL. 2009. Iridescence: views from many angles. *Journal of the Royal Society Interface* **6 Suppl 2**: S107-113.
- Meadows MG, Morehouse NI, Rutowski RL, Douglas JM, McGraw KJ. 2011. Quantifying iridescent coloration in animals: a method for improving repeatability. *Behavioral Ecology and Sociobiology* **65**: 1317-1327.
- Medina I, Newton E, Kearney MR, Mulder RA, Porter WP, Stuart-Fox D. 2018. Reflection of near-infrared light confers thermal protection in birds. *Nature communications* **9**: 1-7.
- Mendes AC, Baran ET, Reis RL, Azevedo HS. 2013. Self-assembly in nature: using the principles of nature to create complex nanobiomaterials. *Wiley Interdiscip Rev Nanomed Nanobiotechnol* **5**: 582-612.
- Mendoza-Galvan A, Del Rio LF, Jarrendahl K, Arwin H. 2018. Graded pitch profile for the helicoidal broadband reflector and left-handed circularly polarizing cuticle of the scarab beetle *Chrysina chrysargyrea*. *Scientific Reports* **8**: 6456.
- Mendoza-Galván A, Järrendahl K, Arwin H. 2019. Graded circular Bragg reflectors: a semi-analytical retrieval of approximate pitch profiles from Mueller-matrix data. *Journal of Optics* **21**.
- Merrick MJ, Smith RJ. 2004. Temperature regulation in burying beetles (*Nicrophorus* spp.: Coleoptera: Silphidae): effects of body size, morphology and environmental temperature. *Journal of Experimental Biology* **207**: 723-733.
- Meyer-Rochow VB, Horridge GA. 1975. The eye of *Anoplognathus* (Coleoptera, Scarabeidae). *Proceedings of the Royal Society of London. Series B: Biological Sciences* **188**.
- Miao J, Wu Y-Q, Li K-B, Jiang Y-L, Gong Z-j, Duan Y, Li T. 2015. Evidence for Visually Mediated Copulation Frequency in the Scarab Beetle *Anomala corpulenta*. *Journal of Insect Behavior* **28**: 175-182.
- Michelson AA. 1911. LXI. On metallic colouring in birds and insects. *The London, Edinburgh, and Dublin Philosophical Magazine and Journal of Science* **21**: 554-567.
- Mitov M. 2017. Cholesteric liquid crystals in living matter. *Soft Matter* **13**: 4176-4209.
- Montgomerie R, Hill G, McGraw K. 2006. Analyzing colors. *Bird coloration* **1**: 90-147.

- Morehouse NI, Rutowski RL. 2009. Comment on "Floral iridescence, produced by diffractive optics, acts as a cue for animal pollinators". *Science* **325**: 1072; author reply 1072.
- Morehouse NI, Vukusic P, Rutowski R. 2007. Pterin pigment granules are responsible for both broadband light scattering and wavelength selective absorption in the wing scales of pierid butterflies. *Proceedings of the Royal Society B: Biological Sciences* **274**: 359-366.
- Mouchet SR, Luke S, McDonald LT, Vukusic P. 2020. Optical costs and benefits of disorder in biological photonic crystals. *Faraday Discussions* **223**: 9-48.
- Mouchet SR, Vukusic P. 2018. Structural colours in lepidopteran scales *Advances in insect physiology*: Elsevier. 1-53.
- Mousseau TA. 1997. Ectotherms Follow the Converse to Bergmann's Rule. *Evolution* **51**.
- Moyroud E, Wenzel T, Middleton R, Rudall PJ, Banks H, Reed A, Mellers G, Killoran P, Westwood MM, Steiner U, Vignolini S, Glover BJ. 2017. Disorder in convergent floral nanostructures enhances signalling to bees. *Nature* **550**: 469-474.
- Muff S, Nilsen EB, O'Hara RB, Nater CR. 2022. Rewriting results sections in the language of evidence. *Trends in Ecology & Evolution* **37**: 203-210.
- Munro JT, Medina I, Walker K, Moussalli A, Kearney MR, Dyer AG, Garcia J, Rankin KJ, Stuart-Fox D. 2019. Climate is a strong predictor of near-infrared reflectance but a poor predictor of colour in butterflies. *Proceedings of the Royal Society B: Biological Sciences* **286**: 20190234.
- Neville AC. 2011. *Biology of the Arthropod Cuticle*. Springer-Verlag Berlin and Heidelberg GmbH & Co. KG: Berlin, Germany.
- Neville AC, Caveney S. 1969. Scarabaeid beetle exocuticle as an optical analogue of cholesteric liquid crystals. *Biological Reviews* **44**: 531-562.
- Ng L, Ospina-Rozo L, Garcia JE, Dyer AG, Stuart-Fox D, Herberstein M. 2022. Iridescence untwined: honey bees can separate hue variations in space and time. *Behavioral Ecology* **33**: 884-891.
- Noh H, Liew SF, Saranathan V, Prum RO, Mochrie SGJ, Dufresne ER, Cao H. 2010. Double scattering of light from Biophotonic Nanostructures with short-range order. *Optics Express* **18**.
- Noh MY, Muthukrishnan S, Kramer KJ, Arakane Y. 2016. Cuticle formation and pigmentation in beetles. *Current opinion in insect science* **17**: 1-9.
- Nordén KK, Eliason CM, Stoddard MC. 2021. Evolution of brilliant iridescent feather nanostructures. *eLife* **10**.
- Norden KK, Faber JW, Babarovic F, Stubbs TL, Selly T, Schiffbauer JD, Peharec Stefanic P, Mayr G, Smithwick FM, Vinther J. 2019. Melanosome diversity and convergence in the evolution of iridescent avian feathers-Implications for paleocolor reconstruction. *Evolution* **73**: 15-27.
- Nørgaard T, Dacke M. 2010. Fog-basking behaviour and water collection efficiency in Namib Desert Darkling beetles. *Frontiers in Zoology* **7**.
- Norris KS. 1967. Color adaptation in desert reptiles and its thermal relationships.
- Nussear KE, Stimandle ET, Tracy RC. 2000. Misconceptions about colour, infrared radiation, and energy exchange between animals and their environments. *Herpetological Journal* **10**: 119-122.
- O'Connor JM, Srivastava SK, Tindale NW, Burnett SE. 2019. From carrion to Christmas beetles: the broad dietary niche of the red fox in a hybrid coastal ecosystem in south-eastern Queensland. *Australian Journal of Zoology* **67**.
- O'Neill EM, Hearn EJ, Cogbill JM, Kajita Y. 2017. Rapid evolution of a divergent ecogeographic cline in introduced lady beetles. *Evolutionary Ecology* **31**: 695-705.
- Odin GP, McNamara ME, Arwin H, Jarrendahl K. 2018. Experimental degradation of helicoidal photonic nanostructures in scarab beetles (Coleoptera:

- Scarabaeidae): implications for the identification of circularly polarizing cuticle in the fossil record. *Journal of the Royal Society Interface* 15.
- Onelli OD, Kamp TV, Skepper JN, Powell J, Rolo TDS, Baumbach T, Vignolini S. 2017. Development of structural colour in leaf beetles. *Scientific Reports* 7: 1373.
- Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pearse W. 2018. caper: Comparative Analyses of Phylogenetics and Evolution in R.
- Osorio D, Ham AD. 2002. Spectral reflectance and directional properties of structural coloration in bird plumage. *Journal of Experimental Biology* 205.
- Osorio D, Vorobyev M. 2008. A review of the evolution of animal colour vision and visual communication signals. *Vision Research* 48: 2042-2051.
- Ospina-Rozo L, Priscilla N, Hutchison J, Meene AVd, Roberts NW, Stuart-Fox D, Roberts A. 2022a. Deconstructed beetles: green composite materials with potential for passive cooling due to high near-infrared reflectivity. *Materials Today Advances Submitted*.
- Ospina-Rozo L, Roberts A, Stuart-Fox D. 2022b. A generalized approach to characterise optical properties of natural objects *Biological Journal of the Linnean Society In Press*.
- Ospina-Rozo L, Subbiah J, Seago A, Stuart-Fox D. 2022c. Pretty cool beetles: can manipulation of visible and near-infrared sunlight prevent overheating? *Integrative Organismal Biology* 4.
- Paradis E, Schliep K. 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in {R}. *Bioinformatics* 35: 526-528.
- Parkash R. 2010. Testing the melanism-desiccation hypothesis: A case study in Darwinian evolution. In: Sharma VP, ed. *Nature at Work: Ongoing Saga of Evolution*. New Delhi: Springer.
- Parkash R, Sharma V, Kalra B. 2009. Impact of body melanisation on desiccation resistance in montane populations of *D. melanogaster*: Analysis of seasonal variation. *Journal of Insect Physiology* 55: 898-908.
- Parker AR, Mckenzie DR, Large MCJ. 1998. Multilayer reflectors in animals using green and gold beetles as contrasting examples. *Journal of Experimental Biology* 201 (Pt 9): 1307-1313.
- Pearson DL. 1988. Biology of Tiger Beetles. *Annual Review of Entomology* 33: 123-147.
- Pearson DL, Knisley CB. 1985. Evidence for Food as a Limiting Resource in the Life Cycle of Tiger Beetles (Coleoptera: Cicindelidae). *Oikos* 45: 161-168.
- Pearson DL, Vogler AP. 2001. *Tiger beetles: the evolution, ecology, and diversity of the cicindelids*. Cornell University Press.
- Pickrell J. 2019. Ghosts of Christmases past: where are all the Christmas beetles? Wildlife: Australian Geographic.
- Pincebourde S, Dillon ME, Woods HA. 2021. Body size determines the thermal coupling between insects and plant surfaces. *Functional Ecology* 35: 1424-1436.
- Pinkert S, Brandl R, Zeuss D. 2017. Colour lightness of dragonfly assemblages across North America and Europe. *Ecography* 40: 1110-1117.
- Poladian L, Wickham S, Lee K, Large MC. 2009. Iridescence from photonic crystals and its suppression in butterfly scales. *Journal of the Royal Society Interface* 6 Suppl 2: S233-242.
- Ponomarenko AG, Prokin AA. 2015. Review of paleontological data on the evolution of aquatic beetles (Coleoptera). *Paleontological Journal* 49.
- Porter WP, Gates DM. 1969. Thermodynamic equilibria of animals with environment. *Ecological Monographs* 39: 227-244.
- Preciado JA, Rubinsky B, Otten D, Nelson B, Martin MC, Greif R. 2002. Radiative Properties of Polar Bear Hair *Advances in Bioengineering*. 57-58.
- Primo JdO, Borth KW, Peron DC, Teixeira VdC, Galante D, Bittencourt C, Anaissi FJ. 2019. Synthesis of green cool pigments (CoxZn1-xO) for application in NIR radiation reflectance. *Journal of Alloys and Compounds* 780: 17-24.

- Prum RO, Quinn T, Torres RH. 2006. Anatomically diverse butterfly scales all produce structural colours by coherent scattering. *Journal of Experimental Biology* **209**: 748-765.
- Prum RO, Torres R. 2003a. Structural colouration of avian skin: convergent evolution of coherently scattering dermal collagen arrays. *Journal of Experimental Biology* **206**: 2409-2429.
- Prum RO, Torres RH. 2003b. A Fourier tool for the analysis of coherent light scattering by bio-optical nanostructures. *Integrative and Comparative Biology* **43**: 591-602.
- Prum RO, Torres RH, Williamson S, Dyck J. 1999a. Two-dimensional Fourier analysis of the spongy medullary keratin of structurally coloured feather barbs. *Proceedings of the Royal Society B: Biological Sciences* **266**.
- Prum RO, Torres RH, Williamson S, Dyck J. 1999b. Two-dimensional Fourier analysis of the spongy medullary keratin of structurally coloured feather barbs. *Proceedings of the Royal Society* **266**.
- Pye JD. 2010. The distribution of circularly polarized light reflection in the Scarabaeoidea (Coleoptera). *Biological Journal of the Linnean Society* **100**: 585-596.
- R Core Team. 2021. R: A language and environment for statistical computing R Foundation for Statistical Computing. 4.1.2 ed. Vienna - Austria.
- Rassart M, Colomer JF, Tabarrant T, Vigneron JP. 2008. Diffractive hygrochromic effect in the cuticle of the hercules beetle *Dynastes hercules*. *New Journal of Physics* **10**.
- Raupach MJ. 2018. Endless forms most beautiful - Ground beetles (Coleoptera, Carabidae) on postal stamps. *Angewandte Carabidologie* **12**: 29-35.
- Reid C. 2020. Christmas beetles gone?: Australian Museum.
- Rommel T, Tammaru T. 2009. Size-dependent predation risk in tree-feeding insects with different colouration strategies: a field experiment. *Journal of Animal Ecology* **78**: 973-980.
- Revell LJ. 2012. phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* **3**: 217-223.
- Ritcher PO. 1958. Biology of Scarabeidae. *Annual Review of Entomology* **3**.
- Rivera J, Murata S, Hosseini MS, Trikanad AA, James R, Pickle A, Yaraghi N, Matsumoto N, Yang W, Parkinson DY, Barnard HS, Zavattieri P, Arakaki A, Kisailus D. 2021. Structural Design Variations in Beetle Elytra. *Advanced Functional Materials* **31**.
- Rivers VZ. 2003. Les Insectes Dans la Tradition Orale/Insects in Oral Literature and Traditions. In: Motte-Florac E and Thomas JMC, eds. *Les Insectes Dans la Tradition Orale/Insects in Oral Literature and Traditions*. France: SELAF/Peeters Publishers. pp. 163-174.
- Roberts NW, Porter ML, Cronin TW. 2011. The molecular basis of mechanisms underlying polarization vision. *Philosophical Transactions of the Royal Society B: Biological Sciences* **366**: 627-637.
- Rogalla S, Patil A, Dhinojwala A, Shawkey MD, D'Alba L. 2021. Enhanced photothermal absorption in iridescent feathers. *Journal of the Royal Society Interface* **18**: 20210252.
- Rosati A, Fedel M, Rossi S. 2021. NIR reflective pigments for cool roof applications: A comprehensive review. *Journal of Cleaner Production* **313**.
- Rothhammer M, Zollfrank C, Busch K, von Freymann G. 2021. Tailored Disorder in Photonics: Learning from Nature. *Advanced Optical Materials* **9**.
- Rozen-Rechels D, Dupoue A, Lourdaïs O, Chamaille-Jammes S, Meylan S, Clobert J, Le Galliard JF. 2019. When water interacts with temperature: Ecological and evolutionary implications of thermo-hydreregulation in terrestrial ectotherms. *Ecology and evolution* **9**: 10029-10043.
- Schaepman-Strub G, Painter T, Huber S, Dangel S, Schaepman, M. E. , Martonchik J, Berendse F. 2004. About the importance of the definition of reflectance

- quantities - Result of case studies. In Proceedings of the XXth ISPRS Congress. 361 - 366.
- Schenk F, Wilts BD, Stavenga DG. 2013. The Japanese jewel beetle: a painter's challenge. *Bioinspiration & biomimetics* 8: 045002.
- Schilman PE, Kaiser A, Lighton JR. 2008. Breathe softly, beetle: continuous gas exchange, water loss and the role of the subelytral space in the tenebrionid beetle, *Eleodes obscura*. *Journal of Insect Physiology* 54: 192-203.
- Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez JY, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A. 2012. Fiji: an open-source platform for biological-image analysis. *Nature methods* 9: 676-682.
- Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature methods* 9: 671-675.
- Schroder-Turk GE. 2020. Quo vadis biophotonics? Wearing serendipity and slow science as a badge of pride, and embracing biology. *Faraday Discussions* 223: 307-323.
- Schroeder TBH, Houghtaling J, Wilts BD, Mayer M. 2018. It's Not a Bug, It's a Feature: Functional Materials in Insects. *Advanced Materials* 30: e1705322.
- Schultz TD. 1986. Role of Structural Colors in Predator Avoidance by Tiger Beetles of the Genus *Cincindela* (Coleoptera: Cincindelidae). *Bulletin of the Entomological Society of America* 32: 142-146.
- Schultz TD, Fincke OM. 2009. Structural colours create a flashing cue for sexual recognition and male quality in a Neotropical giant damselfly. *Functional Ecology* 23: 724-732.
- Schultz TD, Hadley NF. 1987. Structural colors of tiger beetles and their role in heat transfer through the integument. *Physiological Zoology* 60: 737-745.
- Seago AE, Brady P, Vigneron JP, Schultz TD. 2009. Gold bugs and beyond: a review of iridescence and structural colour mechanisms in beetles (Coleoptera). *Journal of the Royal Society Interface* 6(Suppl_2): S165-184.
- Seago AE, Oberprieler R, Saranathan VK. 2019. Evolution of Insect Iridescence: Origins of Three-Dimensional Photonic Crystals in Weevils (Coleoptera: Curculionoidea). *Integrative and Comparative Biology* 59: 1664-1672.
- Seidel M, Reid CAM. 2021. Taxonomic changes resulting from a review of the types of Australian Anoplognathini (Coleoptera: Scarabaeidae: Rutelinae) housed in Swedish natural history collections. *Zootaxa* 4908: zootaxa 4908 4902 4904.
- Shamim G, Ranjan SK, Pandey DM, Ramani R. 2014. Biochemistry and biosynthesis of insect pigments. *European Journal of Entomology* 111: 149-164.
- Shankar R. 2014. *Fundamentals of physics. Mechanics, relativity and thermodynamics*. New Haven : Yale University: United States of America.
- Shanks K, Senthilarasu S, Ffrench-Constant RH, Mallick TK. 2015. White butterflies as solar photovoltaic concentrators. *Scientific Reports* 5: 12267.
- Sharma V, Crne M, Park JO, Srinivasarao M. 2009. Structural origin of circularly polarized iridescence in jeweled beetles. *Science* 325: 449-451.
- Sharma V, Crne M, Park JO, Srinivasarao M. 2014. Bouligand Structures Underlie Circularly Polarized Iridescence of Scarab Beetles: A Closer View. *Materials Today: Proceedings* 1: 161-171.
- Shawkey MD, D'Alba L. 2017. Interactions between colour-producing mechanisms and their effects on the integumentary colour palette. *Philosophical Transactions of the Royal Society B: Biological Sciences* 372.
- Shawkey MD, Hill GE. 2005. Carotenoids need structural colours to shine. *Biology Letters* 1: 121-124.
- Shawkey MD, Igic B, Rogalla S, Goldenberg J, Clusella-Trullas S, D'Alba L. 2017. Beyond colour: consistent variation in near infrared and solar reflectivity in sunbirds (Nectariniidae). *The Science of Nature* 104: 78.

- Shawkey MD, Morehouse NI, Vukusic P. 2009a. A protean palette: colour materials and mixing in birds and butterflies. *Journal of the Royal Society Interface* **6**: S221-231.
- Shawkey MD, Saranathan V, Palsdottir H, Crum J, Ellisman MH, Auer M, Prum RO. 2009b. Electron tomography, three-dimensional Fourier analysis and colour prediction of a three-dimensional amorphous biophotonic nanostructure. *Journal of the Royal Society Interface* **6**: S213-220.
- Shelly TE, Pearson DL. 1978. Size and color discrimination of the robber fly *Efferia tricella* (Diptera: Asilidae) as a predator on tiger Beetles (Coleoptera: Cicindelidae). *Environmental Entomology* **7**: 790-793.
- Shi NN, Tsai C-C, Camino F, Bernard GD, Yu N, Wehner R. 2015. Keeping cool: Enhanced optical reflection and radiative heat dissipation in Saharan silver ants. *Science* **349**.
- Siddique RH, Gomard G, Holscher H. 2015. The role of random nanostructures for the omnidirectional anti-reflection properties of the glasswing butterfly. *Nature communications* **6**: 6909.
- Simpson RK, McGraw KJ. 2018. It's not just what you have, but how you use it: solar-positional and behavioural effects on hummingbird colour appearance during courtship. *Ecology Letters* **21**: 1413-1422.
- Slaton MR, Raymond Hunt E, Smith WK. 2001. Estimating near-infrared leaf reflectance from leaf structural characteristics. *American Journal of Botany* **88**: 278-284.
- Slipinski A, Lawrence J. 2019. *Australian Beetles Volume 2: Archostemata, Myxophaga, Adephaga, Polyphaga (part)*. Csiro Publishing.
- Smith KR, Cadena V, Endler JA, Kearney MR, Porter WP, Stuart-Fox D. 2016a. Color Change for Thermoregulation versus Camouflage in Free-Ranging Lizards. *The American Naturalist* **188**: 668-678.
- Smith KR, Cadena V, Endler JA, Porter WP, Kearney MR, Stuart-Fox D. 2016b. Colour change on different body regions provides thermal and signalling advantages in bearded dragon lizards. *Proceedings of the Royal Society B: Biological Sciences* **283**.
- Smolka J, Baird E, Byrne MJ, el Jundi B, Warrant EJ, Dacke M. 2012. Dung beetles use their dung ball as a mobile thermal refuge. *Current Biology* **22**: R863-864.
- Solano F. 2014. Melanins: skin pigments and much more—types, structural models, biological functions, and formation routes. *New Journal of Science* **2014**: 1-28.
- Srinivasarao M. 1999. Nano-Optics in the Biological World: Beetles, Butterflies, Birds, and Moths. *Chemical Reviews* **99**: 1935-1961.
- Starkey T, Vukusic P. 2013. Light manipulation principles in biological photonic systems. *Nanophotonics* **2**: 289-307.
- Stavenga DG. 2014. Thin Film and Multilayer Optics Cause Structural Colors of Many Insects and Birds. *Materials Today: Proceedings* **1**: 109-121.
- Stavenga DG. 2021. The wing scales of the mother-of-pearl butterfly, *Protogoniomorpha parhassus*, are thin film reflectors causing strong iridescence and polarization. *Journal of Experimental Biology* **224**.
- Stavenga DG, Stowe S, Siebke K, Zeil J, Arikawa K. 2004. Butterfly wing colours: scale beads make white pierid wings brighter. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **271**: 1577-1584.
- Stavenga DG, Wilts BD, Leertouwer HL, Hariyama T. 2011. Polarized iridescence of the multilayered elytra of the Japanese jewel beetle, *Chrysochroa fulgidissima*. *Philosophical Transactions of the Royal Society B: Biological Sciences* **366**: 709-723.
- Steinbauer MJ, Wanjura WJ. 2002. Christmas beetles (*Anoplognathus* spp., Coleoptera: Scarabaeidae) mistake peppercorn trees for eucalypts. *Journal of Natural History* **36**: 119-125.

- Steinbauer MJ, Weir TA. 2007. Summer activity patterns of nocturnal Scarabaeoidea (Coleoptera) of the southern tablelands of New South Wales. *Australian Journal of Entomology* 46: 7-16.
- Stelbrink P, Pinkert S, Brunzel S, Kerr J, Wheat CW, Brandl R, Zeuss D. 2019. Colour lightness of butterfly assemblages across North America and Europe. *Scientific Reports* 9: 1760.
- Stevenson RD. 1985. Body Size and Limits to the Daily Range of Body Temperature in Terrestrial Ectotherms. *The American Naturalist* 125: 102-117.
- Stewart LA, Dixon AFG. 1989. Why Big Species of Ladybird Beetles are Not Melanic. *Functional Ecology* 3.
- Stillwell RC, Morse GE, Fox CW. 2007. Geographic variation in body size and sexual size dimorphism of a seed-feeding beetle. *The American Naturalist* 170: 358-369.
- Stoehr AM. 2006. Costly melanin ornaments: the importance of taxon? *Functional Ecology* 20: 276-281.
- Stoffel MA, Nakagawa S, Schielzeth H. 2017. rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution* 8: 1639-1644.
- Stork NE. 2018. How many species of insects and other terrestrial arthropods are there on Earth? *Annual Review of Entomology* 63: 31-45.
- Stuart-Fox D, Moussalli A. 2009. Camouflage, communication and thermoregulation: lessons from colour changing organisms. *Philosophical Transactions of the Royal Society B: Biological Sciences* 364: 463-470.
- Stuart-Fox D, Newton E, Clusella-Trullas S. 2017. Thermal consequences of colour and near-infrared reflectance. *Philosophical Transactions of the Royal Society B: Biological Sciences* 372: 20160345.
- Stuart-Fox D, Newton E, Mulder RA, D'Alba L, Shawkey MD, Igic B. 2018. The microstructure of white feathers predicts their visible and near-infrared reflectance properties. *PLoS One* 13: e0199129.
- Stuart-Fox D, Ospina-Rozo L, Ng L, Franklin AM. 2020. The Paradox of Iridescent Signals. *Trends in Ecology & Evolution* 36: 187-195.
- Su B, Tian Y, Jiang L. 2016. Bioinspired Interfaces with Superwettability: From Materials to Chemistry. *Journal of the American Chemical Society* 138: 1727-1748.
- Sun J, Bhushan B. 2012. Structure and mechanical properties of beetle wings: a review. *RSC Advances* 2.
- Sun J, Cai F, Tao D, Ni Q, Fu Y. 2021. Enhanced Thermal Insulation of the Hollow Glass Microsphere/Glass Fiber Fabric Textile Composite Material. *Polymers* 13: 505.
- Sun M, Liang A, Watson GS, Watson JA, Zheng Y, Jiang L. 2012. Compound microstructures and wax layer of beetle elytral surfaces and their influence on wetting properties. *PLoS One* 7: e46710.
- Temple SE, How MJ, Powell SB, Gruev V, Marshall NJ, Roberts NW. 2021. Thresholds of polarization vision in octopuses. *Journal of Experimental Biology* 224.
- Teyssier J, Saenko SV, van der Marel D, Milinkovitch MC. 2015. Photonic crystals cause active colour change in chameleons. *Nature communications* 6: 6368.
- Thomas DB, Seago A, Robacker DC. 2007. Reflections on Golden Scarabs. *American Entomologist* 53: 224-230.
- Tomoyasu Y, Arakane Y, Kramer KJ, Denell RE. 2009. Repeated co-options of exoskeleton formation during wing-to-elytron evolution in beetles. *Current Biology* 19: 2057-2065.
- Tsai CC, Childers RA, Nan Shi N, Ren C, Pelaez JN, Bernard GD, Pierce NE, Yu N. 2020. Physical and behavioral adaptations to prevent overheating of the living wings of butterflies. *Nature communications* 11: 551.
- Turner JS, Lombard AT. 1990. Body color and body temperature in white and black Namib desert beetles. *Journal of Arid Environments* 19: 303-315.

- Umbers KD, Herberstein ME, Madin JS. 2013. Colour in insect thermoregulation: empirical and theoretical tests in the colour-changing grasshopper, *Kosciuscola tristis*. *Journal of Insect Physiology* 59: 81-90.
- Van de Kamp T, Hartmut G. 2010. On the Architecture of Beetle Elytra. *Entomologie heute* 22: 191-204.
- Vargas WE, Avendano E, Hernandez-Jimenez M, Azofeifa DE, Libby E, Solis A, Barboza-Aguilar C. 2018. Photonic crystal characterization of the cuticles of *Chrysina chrysargyrea* and *Chrysina optima* jewel scarab beetles. *Biomimetics* 3.
- Vigneron JP, Pasteels JM, Windsor DM, Vertesy Z, Rassart M, Seldrum T, Dumont J, Deparis O, Lousse V, Biro LP, Ertz D, Welch V. 2007. Switchable reflector in the Panamanian tortoise beetle *Charidotella egregia* (Chrysomelidae: Cassidinae). *Physical Review E* 76: 031907.
- Vignolini S, Moyroud E, Glover BJ, Steiner U. 2013. Analysing photonic structures in plants. *Journal of the Royal Society Interface* 10: 20130394.
- Vinther J. 2020. Reconstructing Vertebrate Paleocolor. *Annual Review of Earth and Planetary Sciences* 48: 345-375.
- Vogler AP, Kelley KC. 1998. Covariation of Defensive Traits in Tiger Beetles (Genus *Cicindela*): A Phylogenetic Approach Using mtDNA. *Evolution* 52: 529-538.
- Vukusic P, Sambles JR. 2003. Photonic structures in biology. *Nature* 424.
- Vukusic P, Sambles JR, Lawrence CR. 2004. Structurally assisted blackness in butterfly scales. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 271 Suppl 4: S237-239.
- Vukusic P, Sambles JR, Lawrence CR, Wootton RJ. 2002. Limited-view iridescence in the butterfly *Ancyluris meliboeus*. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 269: 7-14.
- Vukusic P, Stavenga DG. 2009. Physical methods for investigating structural colours in biological systems. *Journal of the Royal Society Interface* 6: S133-148.
- Wagner T, Neinhuis C, Barthlott W. 1996. Wettability and contaminability of insect wings as a function of their surface sculptures. *Acta Zoologica* 77: 213-225.
- Waldron SJ, Endler JA, Valkonen JK, Honma A, Dobler S, Mappes J. 2017. Experimental evidence suggests that specular reflectance and glossy appearance help amplify warning signals. *Scientific Reports* 7: 1-10.
- Walsberg GE. 1991. Thermal effects of seasonal coat change in three subarctic mammals. *Journal of Thermal Biology* 16: 291-296.
- Wang L-Y, Franklin AM, Hugall AF, Medina I, Stuart-Fox D. 2022a. Disentangling thermal from alternative drivers of reflectance in jewel beetles: a macroecological study. *Global Ecology and Biogeography* Forthcoming.
- Wang L, Urbas AM, Li Q. 2020. Nature-Inspired Emerging Chiral Liquid Crystal Nanostructures: From Molecular Self-Assembly to DNA Mesophase and Nanocolloids. *Advanced Materials* 32: e1801335.
- Wang LY, Franklin AM, Black JR, Stuart-Fox D. 2021. Heating rates are more strongly influenced by near-infrared than visible reflectance in beetles. *Journal of Insect Physiology* 224.
- Wang LY, Stuart-Fox D, Walker G, Roberts NW, Franklin AM. 2022b. Insect visual sensitivity to long wavelengths enhances colour contrast of insects against vegetation. *Scientific Reports* 12: 982.
- Westgate M, Stevenson M, Newman P, Raymond B, VanDerWal J, Belbin L. 2022. ALA4R: Atlas of Living Australia (ALA) Data and Resources in R. R package version 1.9.1.
- White TE. 2018. Illuminating the Evolution of Iridescence. *Trends in Ecology & Evolution* 33: 374-375.
- White TE. 2020. Structural colours reflect individual quality: a meta-analysis. *Biology Letters* 16.

- White TE, Zeil J, Kemp DJ. 2015. Signal design and courtship presentation coincide for highly biased delivery of an iridescent butterfly mating signal. *Evolution* **69**: 14-25.
- Wigglesworth VB. 1990. The properties of the lining membrane of the insect tracheal system. *Tissue and Cell* **22**.
- Willmer PG, Unwin DM. 1981. Field analyses of insect heat budgets: Reflectance, size and heating rates. *Oecologia* **50**: 250-255.
- Wilts BD, Leertouwer HL, Stavenga DG. 2009. Imaging scatterometry and microspectrophotometry of lycaenid butterfly wing scales with perforated multilayers. *Journal of the Royal Society Interface* **6**: S185-192.
- Wilts BD, Pirih P, Arikawa K, Stavenga DG. 2013. Shiny wing scales cause spec(tac)ular camouflage of the angled sunbeam butterfly, *Curetis acuta*. *Biological Journal of the Linnean Society* **109**: 279-289.
- Wilts BD, Sheng X, Holler M, Diaz A, Guizar-Sicairos M, Raabe J, Hoppe R, Liu SH, Langford R, Onelli OD, Chen D, Torquato S, Steiner U, Schroer CG, Vignolini S, Sepe A. 2018. Evolutionary-Optimized Photonic Network Structure in White Beetle Wing Scales. *Advanced Materials* **30**: e1702057.
- Wittkopp PJ, Beldade P. 2009. Development and evolution of insect pigmentation: genetic mechanisms and the potential consequences of pleiotropy. *Seminars in cell & developmental biology*: Elsevier, 65-71.
- Wu K, Song Z, Zhang S, Ni Y, Cai S, Gong X, He L, Yu S-H. 2020. Discontinuous fibrous Bouligand architecture enabling formidable fracture resistance with crack orientation insensitivity. *Proceedings of the National Academy of Sciences* **117**: 15465-15472.
- Xie D, Yang Z, Liu X, Cui S, Zhou H, Fan T. 2019. Broadband omnidirectional light reflection and radiative heat dissipation in white beetles *Goliathus goliatus*. *Soft Matter* **15**: 4294-4300.
- Xing S, Bonebrake TC, Ashton LA, Kitching RL, Cao M, Sun Z, Ho JC, Nakamura A. 2018. Colors of night: climate-morphology relationships of geometrid moths along spatial gradients in southwestern China. *Oecologia* **188**: 537-546.
- Yang R, Zaheri A, Gao W, Hayashi C, Espinosa HD. 2017. AFM Identification of Beetle Exocuticle: Bouligand Structure and Nanofiber Anisotropic Elastic Properties. *Advanced Functional Materials* **27**: 1603993.
- Yang Z, Dai Z, Guo C. 2010. Morphology and mechanical properties of *Cybister* elytra. *Chinese Science Bulletin* **55**: 771-776.
- Yoshioka S, Kinoshita S. 2005. Structural or pigmentary? Origin of the distinctive white stripe on the blue wing of a Morpho butterfly. *Proceedings of the Royal Society B: Biological Sciences* **273**: 129-134.
- Yu Y, Wen L, Song S, Chen Q. 2014. Transmissive/Reflective Structural Color Filters: Theory and Applications. *Journal of Nanomaterials* **2014**: 1-17.
- Yu Z, Ji Y, Bourg V, Bilgen M, Meredith JC. 2020. Chitin- and cellulose-based sustainable barrier materials: a review. *Emergent Materials* **3**: 919-936.
- Zaheri A, Fenner JS, Russell BP, Restrepo D, Daly M, Wang D, Hayashi C, Meyers MA, Zavattieri PD, Espinosa HD. 2018. Revealing the Mechanics of Helicoidal Composites through Additive Manufacturing and Beetle Developmental Stage Analysis. *Advanced Functional Materials* **28**: 1803073.
- Zeil J, Ribi WA, Narendra A. 2014. Polarisation Vision in Ants, Bees and Wasps *Polarized Light and Polarization Vision in Animal Sciences*. 41-60.
- Zeuss D, Brandl R, Brandle M, Rahbek C, Brunzel S. 2014. Global warming favours light-coloured insects in Europe. *Nature communications* **5**: 3874.
- Zhang F, Kearns SL, Orr PJ, Benton MJ, Zhou Z, Johnson D, Xu X, Wang X. 2010. Fossilized melanosomes and the colour of Cretaceous dinosaurs and birds. *Nature* **463**: 1075-1078.
- Zhang W, Zhao X, Xiong Y, Wang W, Zhang D. 2022. Achieve structural white inspired from quasiordered microstructures in *Morpho theseus*. *PREPRINT*.

- Zhao D, Yang H, Ma Z, Zhou W. 2011. Polarization independent broadband reflectors based on cross-stacked gratings. *Optics Express* **19**.
- Zink R, Remsen Jr J. 1986. Evolutionary processes and patterns of geographic variation in birds.
- Zobl S, Wilts BD, Salvenmoser W, Pölt P, Gebeshuber IC, Schwerte T. 2020. Orientation-dependent reflection of structurally coloured butterflies. *Biomimetics* **5**: 5.
- Zverev V, Kozlov MV, Forsman A, Zvereva EL. 2018. Ambient temperatures differently influence colour morphs of the leaf beetle *Chrysomela lapponica*: Roles of thermal melanism and developmental plasticity. *Journal of Thermal Biology* **74**: 100-109.

Appendix to Chapter 2

A Generalized Approach to Characterize Optical Properties Of Natural Object

Supplementary figures

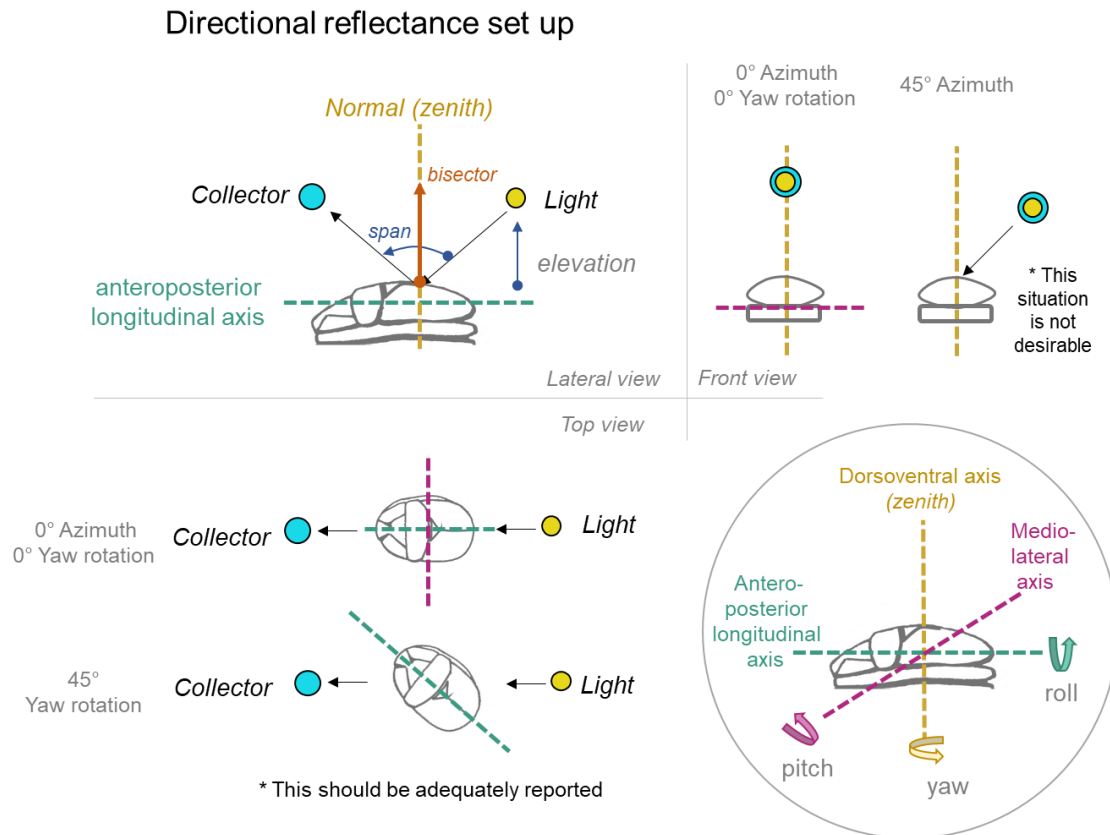


Figure A-1. 3D planes describing our goniometer set up to measure directional reflectance

A goniometer allows different combinations of angles for the incident light and the collector. We use the axes of a beetle to illustrate the 3 dimensions (circle). In our set up, the light and the collector are always aligned in the plane of the anteroposterior longitudinal axis of the sample, which means they are always at 0° azimuth. The position of the sample must be adjusted at the optimal distance for the light and collector to be in focus, which was signalled by the focus plane of a customized camera attached to the collector. The changes in the bisector and span angle cause changes in the **elevation** of both light and collector. We adjust the rotation in **pitch** and **roll** to ensure that the sample is as flat as possible. Changing the **azimuth** angle (front view) is not desirable because it may result in an angled surface or alignment issues. Changing the **yaw** rotation may be informative and should always be reported, since the optical properties may differ with different yaw angles in non-symmetric structures. In our experiment we measured all our samples at 0° yaw.

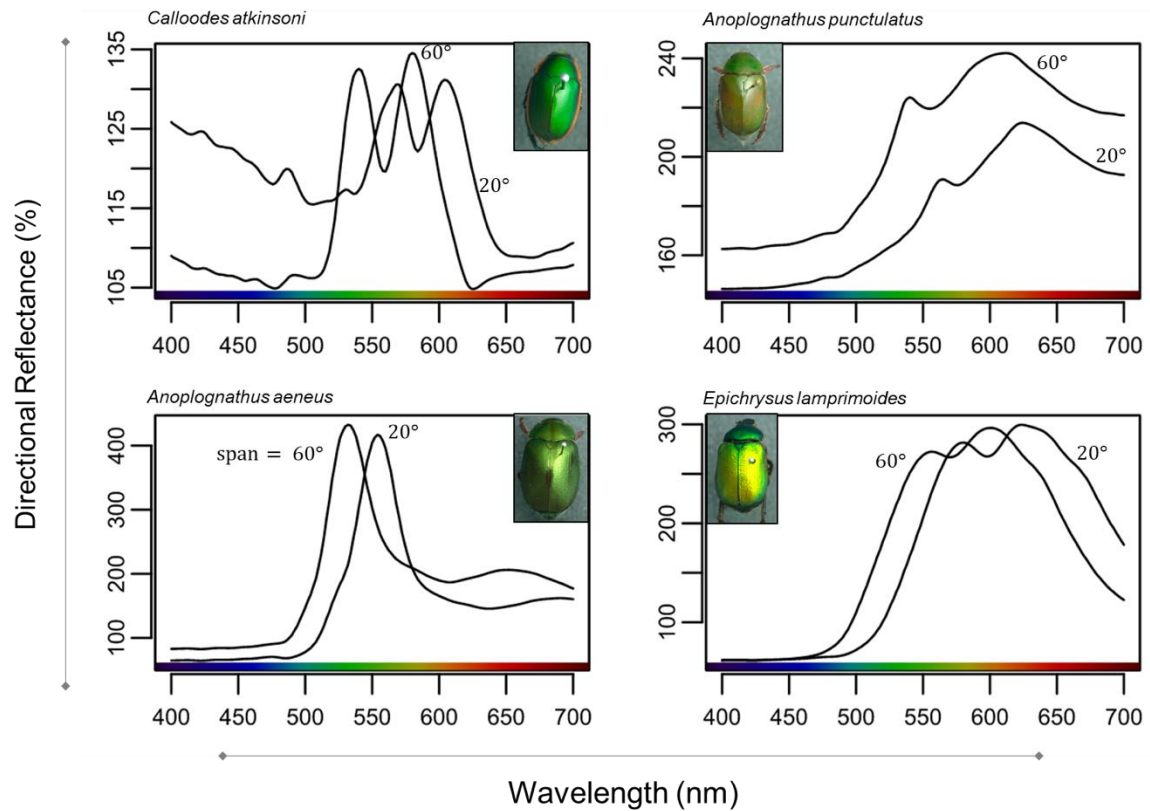


Figure A-2. Green Diversity

These four green beetles have very different spectral profiles in the visible directional reflectance. In all cases, the geometries compared are span at 20° and span at 60° in the fixed bisector set. The four beetles showed a shift towards shorter wavelengths with the increase in span angle, which corresponds to iridescence.

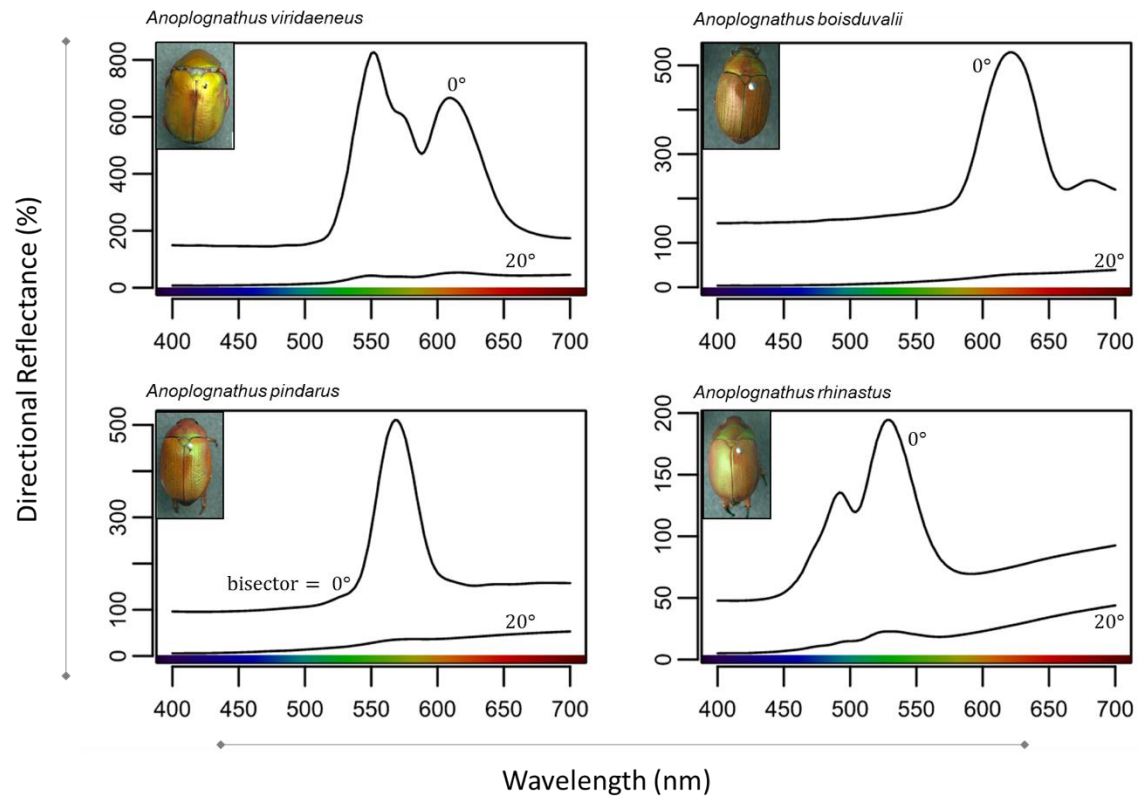


Figure A-3. Sheen

These four green beetles have a brown background with a topcoat shiny sheen which is only visible at certain angles. Thus, in order to compare them, we used the fixed span set, with the bisector at 0° (centered in the normal) and bisector at 20°. The optical properties of the sheen are detectable only when measuring at the specular angle, and they vary considerably between species. The measurements away from the normal describe the properties of the brown background.

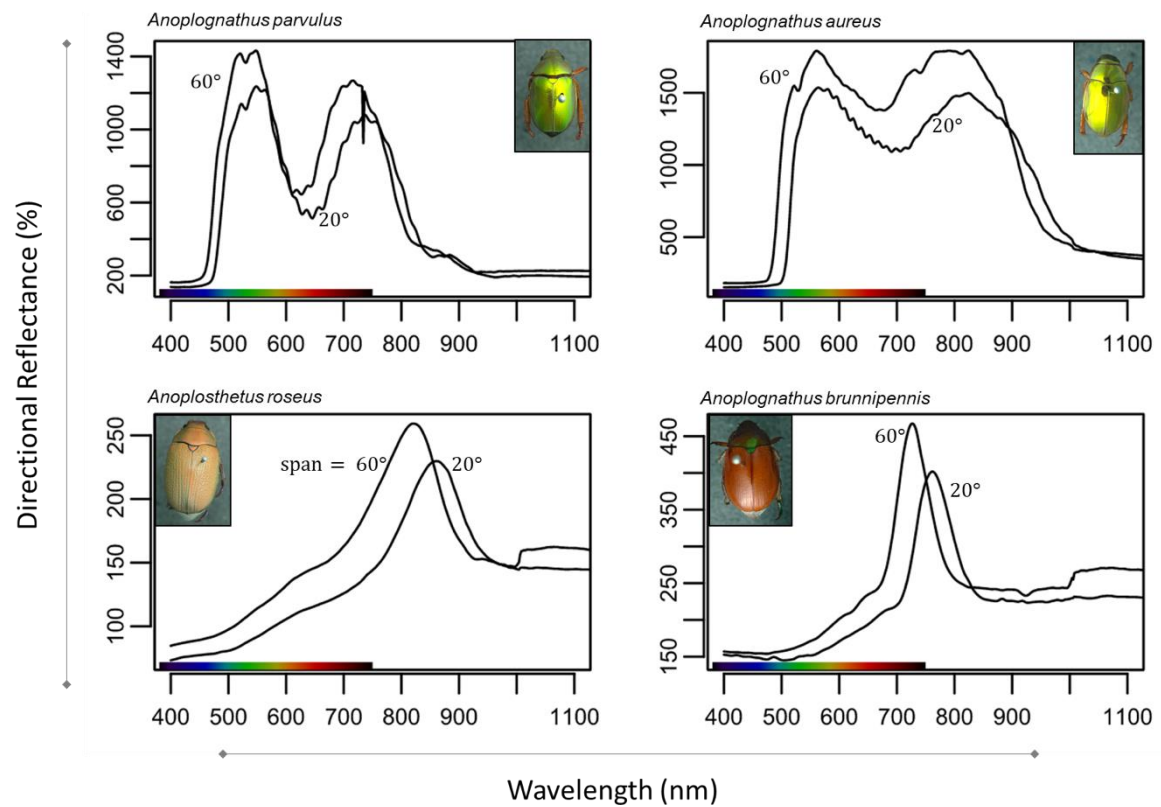


Figure A-4. Iridescent NIR

These four beetles have defined peaks in the near infrared (NIR) and all of them showed a shift towards shorter wavelengths with the increase in span angle, which corresponds to iridescence. In all cases, the geometries compared are span at 20° and span at 60° in the fixed bisector set.

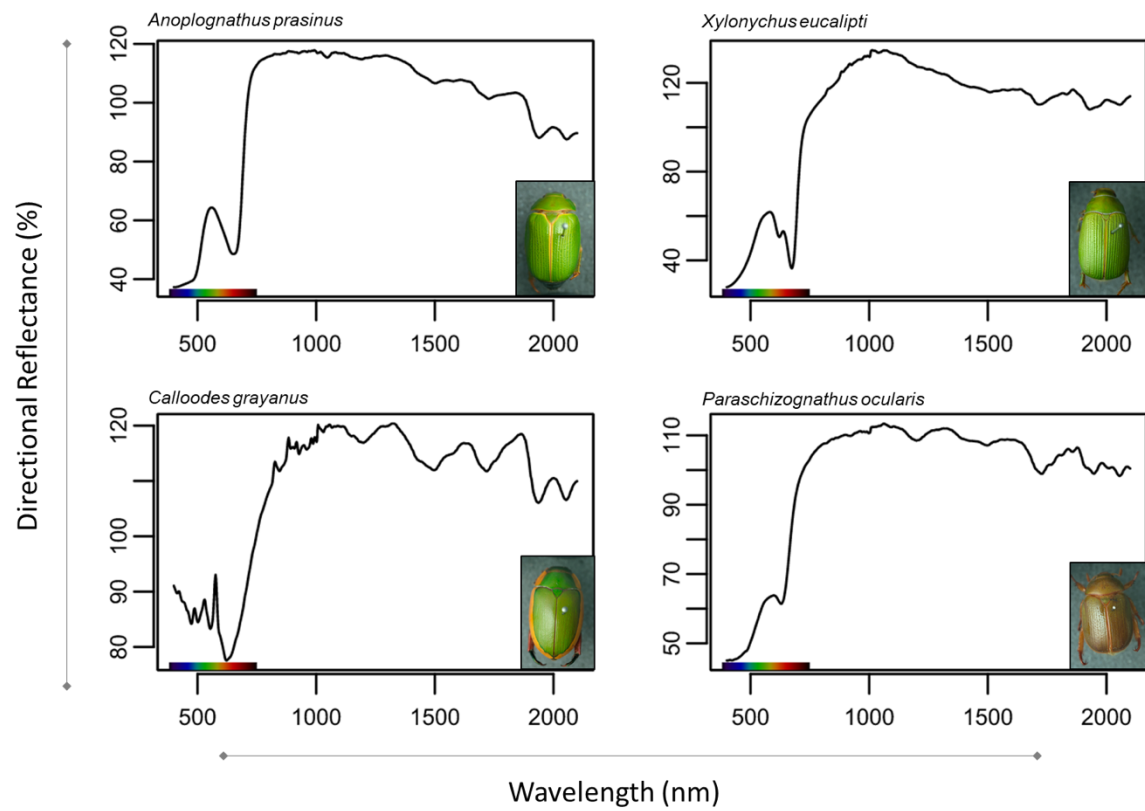


Figure A-5. Diffuse NIR

These four beetles have a high near infrared (NIR) reflectance with a plateau. In these cases, the geometry was the span at 20° in the fixed bisector set.

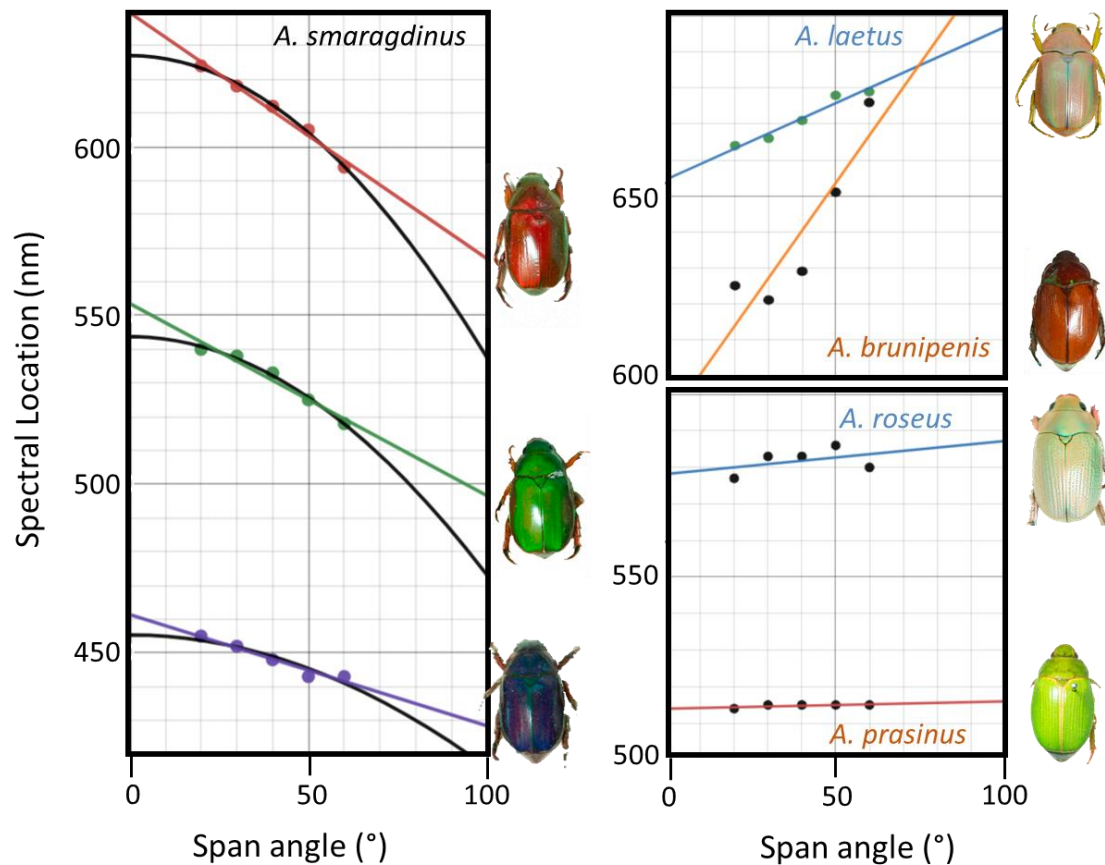


Figure A-6. Examples of the application of linear vs. sinusoidal models

For *A. smaragdinus* (red, green and blue morph) the two models behave similarly in the range of angles that we studied. In span angles smaller than 20° the linear model overestimates the spectral location, therefore overestimating the iridescence. However, for species with a red shift (top right) or without any angle dependency in the spectra (bottom right), a sinusoidal model is not able to describe the pattern. The red shift in *A. laetus* and *A. brunipennis* is produced by the presence of an iridescent peak in near infrared (see Figure 2-3 and Figure 2-4). Although the pattern in NIR could be explained by a sinusoid, not all studies are able to or interested in measuring NIR. Instead, linear models allow the simplest and most easily interpretable comparison between beetles with different degrees of angle dependent shifting of the spectra.

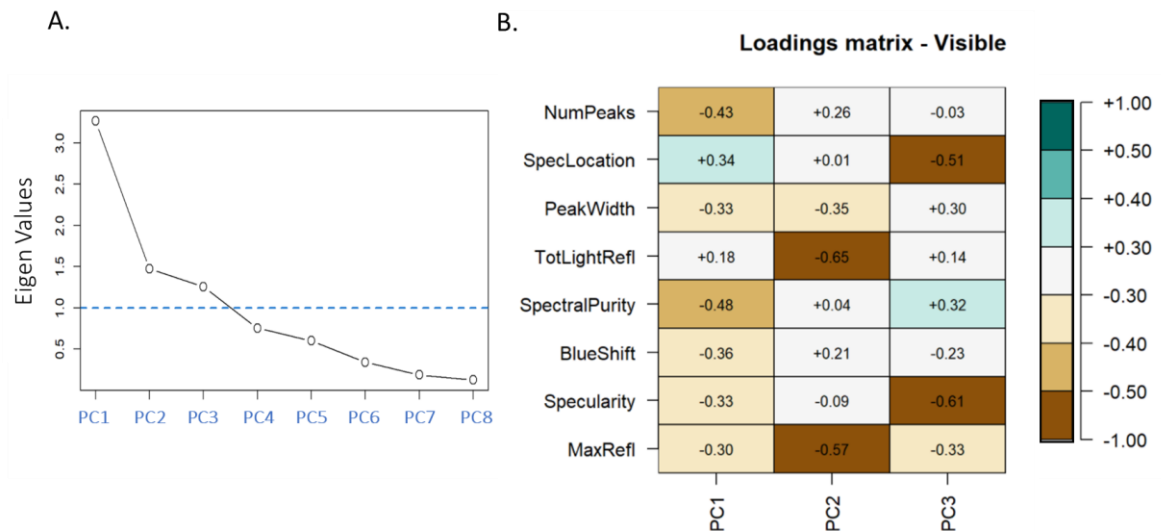


Figure A-7. PCA results for optical effects in visible light

A) Scree plot. Only the first three principal components have eigen values >1, so we only considered these three. **B)** Loadings Matrix. Correlations between spectral parameters and the first three principal components. Scale bar: Significant correlations (beyond -0.3 to +0.3 range) are shaded in colours. PC1 correlates negatively with spectral purity, number of peaks and iridescence (as blue shift). PC2 correlates strongly with the total light reflected and the maximum directional reflectance. PC3 correlates positively with specularity and spectral location but negatively with peak width.

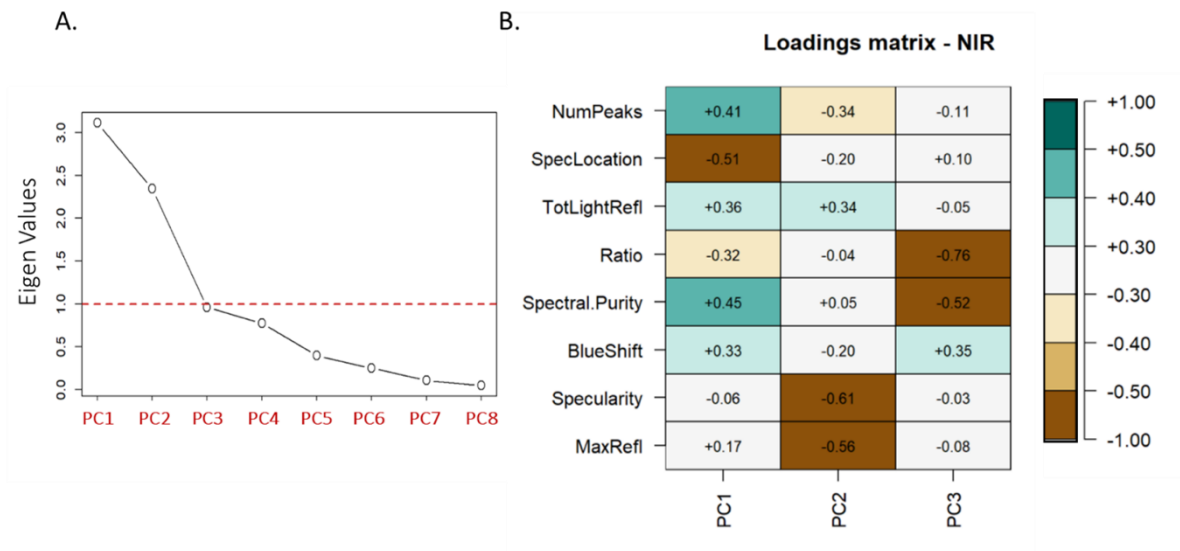


Figure A-8. PCA results for optical effects in near infrared light

A) Scree plot. Only the first two principal components have eigen values >1, so we only considered these two. **B)** Loadings Matrix. Correlations between spectral parameters and the first 3 principal components (due to the eigen value of PC3 being close to 1). Scale bar: Significant correlations (beyond -0.3 to +0.3 range) are shaded in colours. PC1 correlates positively with the number of peaks, spectral purity and iridescence (as blue shift); and negatively with spectral location and NIR/VIS ratio. PC2 correlates positively with the total light reflected and negatively with specularity and the maximum directional reflectance.

Supplementary tables

Table A-1. Specimens used in this experiment

All specimens were obtained from the Australian National Insect Collection (ANIC). Accession number or label information for unregistered specimens are listed.

Species	Code	ANIC label information
<i>Anomala antiqua</i>	anom	12.21S 130.42E Casuarina Beach, NT. 10 Km NNE of Darwin 7-11-72. Rainforest lightly beach. E. Britton
<i>Anoplognathus aeneus</i>	neus	AN25 - 000105
<i>Anoplognathus aureus</i>	aurs	AN25 - 000244
<i>Anoplognathus boisduvalii</i>	boid	AN25 - 000326
<i>Anoplognathus brunnipennis</i>	brun	AN25 - 000612
<i>Anoplognathus chloropyrus</i>	clor	AN25 - 002572
<i>Anoplognathus concolor</i>	conc	AN25 - 002674
<i>Anoplognathus macleayi</i>	macl	AN25 - 003228
<i>Anoplognathus pallidicollis</i>	pali	AN25 - 008590
<i>Anoplognathus parvulus</i>	pvul	AN25 - 008709
<i>Anoplognathus pindarus</i>	pind	AN25 - 008997
<i>Anoplognathus porosus</i>	pora	AN25 - 009349
<i>Anoplognathus porosus</i>	porb	AN25 - 009641
<i>Anoplognathus prasinus</i>	prsi	AN25 - 010039
<i>Anoplognathus punctulatus</i>	punc	AN25 - 010186
<i>Anoplognathus rhinastus</i>	rina	AN25 - 010272
<i>Anoplognathus rothschildi</i>	roci	AN25 - 010586
<i>Anoplognathus rugosus</i>	ruco	AN25 - 010749
<i>Anoplognathus smaragdinus</i>	smgg	AN25 - 010857
<i>Anoplognathus smaragdinus</i>	smgr	AN25 - 010756
<i>Anoplognathus smaragdinus</i>	smgp	AN25 - 010755
<i>Anoplognathus viridaeneus</i>	vrdi	AN25 - 011299
<i>Anoplognathus viriditarsis</i>	tars	AN25 - 011524
<i>Anoplosthetus laetus</i>	lats	Ewan Rd QLD c. 8 Km W of Paluma 4 jan 1967 J. B. Brooks

Species	Code	ANIC label information
<i>Anoplosthetus opalinus</i>	opal	Collected in Perth
<i>Anoplosthetus roseus</i>	rose	11.09 S 132.09 E Black Point Coburg Pen N.T. 8 feb 1977. R. Lewis
<i>Calloodes atkinsoni</i>	atki	Mr. Mt. Molloy N. Q. 16. 41 S 145.20E at Station Ck. 2 Jan 1970 at 168ight J. G. Brooks
<i>Calloodes frenchi</i>	fchi	11.45S 142.35E Heathlands QLD. 15 - 26 Jan 1992 T.A. Weir ID Naumann at light
<i>Calloodes grayanus</i>	gray	12.19S 133.19E Nabarlek N.T. 15 Apr 1933 Melanie Webb
<i>Calloodes rayneri</i>	rayn	16.31S 145.04E 7Km WbyN of Mt Carbipe QLD 24 No. 1981 J. Balderson
<i>Epichrysus lamprimoides</i>	ecry	Albany W.A. Jan 1935 K. K. Norris
<i>Paraschizognathus ocularis</i>	psch	Dorrigo N. P. NSW dec 1967 E. Matthews
<i>Repsimus manicatus manicatus</i>	reps	Wallambine Ck. Rd. crossing 20 mi N of Wiseman's Ferry, NSW 29 nov 1967 Britton and Misko
<i>Repsimus manicatus montanus</i>	repb	Casuarina Sand A.C.T. 25 Dec 1977 D. P. Came
<i>Xylonychus eucalypti</i>	xyle	State forest Gibraltar Range. Oct 1963 C. W Frazier

Table A-2. Repeatability for basic parameters used in describing spectral curves

We considered a representative sample of 7 species: *A. aureus*, *P. ocularis*, *C. frenchi*, *A. concolor*, *A. viriditarsis*, *A. laetus* and *A. prasinus*. We calculated the repeatability for spectral location and reflectance for the hemispherical reflectance (VIS and NIR) and directional reflectance, since these two parameters are either used directly in our analysis (in parameters such as spectral location or total hemispherical reflectance) or are required to calculate angle dependent parameters (i.e spectral location is required to calculate iridescence and mean reflectance is required to calculate specularly). For hemispherical reflectance we compared 4 repetitions per species (7 groups x 4 repetitions = 28 observations), while for directional reflectance we compared 3 repetitions per species on each of the angle combinations in the fixed bisector set (7 species x 5 angles = 35 groups) and 3 repetitions per species on each of the angle combinations in the fixed span set (7 species x 7 angles = 49 groups, 147 observations). We estimated repeatability using a bootstrapping tool for gaussian data and posteriorly calculating the percentage of variance explained by the groups (repeatability estimate and its standard error reported in table S3), with p-values based on a likelihood ratio test (Table S3) This analysis was conducted with the package “rptR” in R. Repeatability was high for all the studied parameters indicating that our measurements are reliable.

Technique	Hemispherical Reflectance				Directional Reflectance	
Spectral band	VIS	VIS	NIR	NIR	VIS	VIS
Parameter	Spectral Location (nm)	Total Reflectance (%)	Spectral Location (nm)	Total Reflectance (%)	Spectral Location (nm)	Mean Reflectance (%)
Repeatability	0.825	0.977	0.905	0.983	0.998	0.92
SE Repeatability	0.135	0.028	0.095	0.026	0.001	0.02
<i>p-value</i>	2.44E-07	2.10E-16	5.10E-10	8.04E-18	1.19E-87	1.29E-44
Groups	7	7	7	7	35	49
Observations	28	28	28	28	105	147

Table A-3. Variability in the parameters of hemispherical reflectance spectra.

We calculated the variability as the standard deviation in nm for the spectral location parameter, and the coefficient of variation (standard deviation normalized by the mean of the parameter) “CV” in %. The variability is relatively low as shown by the median of the variation for each parameter.

Hemispherical Reflectance				
	VIS	VIS	NIR	NIR
	Total Reflectance	Spectral Location	Total Reflectance	Spectral Location
	(CV %)	(sd in nm)	(CV %)	(sd in nm)
<i>A. aureus</i>	8.75	1.91	7.23	7.55
<i>A. concolor</i>	7.82	2.87	7.13	1.73
<i>C. frenchi</i>	25.01	3.87	11.23	1.73
<i>A. laetus</i>	9.39	4.97	3.34	1.29
<i>A. prasinus</i>	30.9	2.06	14.29	0.58
<i>P. ocularis</i>	5.32	13.89	8.48	6.22
<i>A. viriditarsis</i>	28.6	2.22	6.13	14.55
<i>Median</i>	9.39	2.87	7.23	1.73

Table A-4. Variability in spectral location

Standard deviation in spectral location of the directional reflectance spectra in different span angles in nm. The variation is generally low (median = 1.15 nm, min = 0 nm, max = 8.54 nm), so the parameter is reliable.

	Span Angle (Fixed Bisector Set)				
	20°	30°	40°	50°	60°
<i>A. aureus</i>	1.53	1.15	1	2.08	1.15
<i>A. concolor</i>	2.52	2.52	6.56	8.54	4.16
<i>C. frenchi</i>	0	0	0.58	0.58	0.58
<i>A. laetus</i>	1.53	1.53	1.15	1.15	0.58
<i>A. prasinus</i>	1	0	0.58	1.53	1.53
<i>P. ocularis</i>	1	1	1	1	0.58
<i>A. viriditarsis</i>	4.93	1.53	2.08	3.51	2.08

Table A-5. Variability in mean reflectance

Coefficient of variation (%) of the mean reflectance of the directional reflectance spectra in different bisector angles in nm.

	Bisector Angle (Fixed Span Set)						
	-10°	-20°	-30°	0°	10°	20°	30°
<i>A. aureus</i>	7.92	62.42	14.67	29.78	28.06	7.43	6.82
<i>A. concolor</i>	6.95	8.62	1.23	16.35	1.61	1.73	2.05
<i>C. frenchi</i>	18.15	15.89	25.44	5.65	27	22.88	9.34
<i>A. laetus</i>	11.43	1.75	1.58	3.25	7.4	8.65	0.87
<i>A. prasinus</i>	1.64	2.26	4	4.27	5.98	8.51	16.65
<i>P. ocularis</i>	14.65	3.69	4.32	4.19	6.94	3.29	0.27
<i>A. viriditarsis</i>	21.74	7.56	1.8	3.89	5.99	4.56	1.05

Table A-6. Comparison between cosine and linear models to describe iridescence

Cosine was used in (Gruson *et al.*, 2019a). Y is the spectral location at a given angle (nm) and x is the span angle (degrees). For the beetles with blue shift, the two models behave similarly. Although the intercept of the two models differs in 37.23 nm approx., parameters b and d are correlated (Pearson Correlation Coefficient 0.925. interval: 0.84 ; 0.97. $t = 12.24$, $df = 25$, $p\text{-value} = 4.6 \times 10^{-12}$). A larger blue shift (iridescence) is defined by a larger b (inverse of the period in the sinusoid) and larger absolute value of d (linear slope). In other words, a smaller period in the sinusoid is equivalent to a more negative slope in the linear model. In our sample, the average blue shift according to the linear models was 0.57 nm per one degree increase in span, the maximum was 0.76 nm per degree and the minimum was 0.33 nm per degree.

Species	Sinusoidal Model			Linear Model		
	$Y = a \cos (b * (x/2))$			$Y = c + d x$		
	a	b	R^2	c	d	R^2
<i>A. laetus</i>				654.8	+ 0.42	0.9525
<i>A. opalinus</i>				523.8	- 0.02	0.0027
<i>A. roseus</i>				578.2	+ 0.09	0.1731
<i>E. lamprimoides</i>	545.94	0.5987	0.9986	556.2	- 0.59	0.9745
<i>P. ocularis</i>				517.8	+ 0.05	0.8929
<i>C. atkinsoni</i>	552.27	0.6071	0.9954	563.2	- 0.62	0.9928
<i>C. frenchi</i>	614.85	0.6032	0.9974	626.8	- 0.68	0.9897
<i>C. grayanus</i>	569.15	0.6693	0.9935	582.2	- 0.76	0.9519
<i>C. rayneri</i> *	568.13	0.4956	0.6737	573.8	- 0.38	0.5324
<i>A. antiqua</i>	611.71	0.5981	0.9979	623.2	- 0.66	0.9749
<i>R. manicatus</i>	439.05	0.5498	0.9909	446	- 0.40	0.9639
<i>R. manicatus</i>	543.97	0.6184	0.9991	555	- 0.63	0.9854
<i>A. aeneus</i>	536.46	0.5596	0.9976	545.4	- 0.51	0.9852
<i>A. aureus</i>	519.46	0.5688	0.9977	528.4	- 0.51	0.9852
<i>A. boisduvali</i>	601.15	0.6201	0.9993	613.4	- 0.70	0.9855
<i>A. brunnipennis</i>				587.6	+ 1.32	0.8206
<i>A. chloropyrus</i>	557.26	0.6216	0.9999	568.6	- 0.65	0.9798
<i>A. concolor</i>				572.4	+ 0.02	0.0213
<i>A. macleayi</i>	443.09	0.5606	0.0097	450.4	- 0.42	0.9735

Species	Sinusoidal Model			Linear Model		
	$Y = a \cos (b * (x/2))$			$Y = c + d x$		
<i>A. pallidicollis</i>	582.09	0.1931	0.398	583	- 0.06	0.3214
<i>A. parvulus</i>	493.12	0.5541	0.9959	501.2	- 0.46	0.9851
<i>A. pindarus</i>	554.81	0.6219	0.9980	566.2	- 0.65	0.9853
<i>A. porosus</i>	590.15	0.6259	0.9993	602.4	- 0.70	0.9855
<i>A. porosus</i>	615.85	0.6185	0.9987	628.2	- 0.71	0.9754
<i>A. prasinus</i>				514	+ 0.02	0.5
<i>A. punctulatus</i>	553.86	0.6318	0.9987	565.6	- 0.67	0.987
<i>A. rhinastus</i>	472.79	0.5039	0.9834	479.4	- 0.37	0.9989
<i>A. rothschildi</i>	537.62	0.4494	0.9987	543.4	- 0.33	0.9829
<i>A. rugosus</i>	611.91	0.4617	0.8769	619.4	- 0.41	0.9236
<i>A. smaragdinus</i>	627.04	0.6203	0.9966	693.8	- 0.73	0.9905
<i>A. smaragdinus</i>	543.75	0.5911	0.9926	553.6	- 0.57	0.959
<i>A. smaragdinus</i>	455.35	0.4795	0.8942	461.4	- 0.33	0.9486
<i>A. viridaeneus</i>	536.57	0.627	0.9969	547.8	- 0.64	0.9875
<i>A. viriditarsis</i>	556.97	0.5892	0.9755	567.4	- 0.59	0.9756
<i>X. eucalypti</i>				502	+ 0.07	0.7206

Table A-7. Percentage of variance explained by the first 3 components

The cumulative proportion of variance explained is 74.8% (highlighted in blue)

	PC1	PC2	PC3
Standard deviation	1.865	1.213	1.119
Proportion of variance	0.408	0.184	0.157
Cumulative proportion	0.408	0.592	0.748

Table A-8. Percentage of variance explained by the first 3 components

The cumulative proportion of variance explained by PC1 and PC2 is 68.2% (highlighted in red).

	PC1	PC2	PC3
Standard deviation	1.764	1.531	9.979
Proportion of variance	0.389	0.293	0.120
Cumulative proportion	0.369	0.682	0.802

Appendix to Chapter 3

Bilayered Composite Materials Produce Green
Coloration with Remarkably High Near-infrared
Reflectance

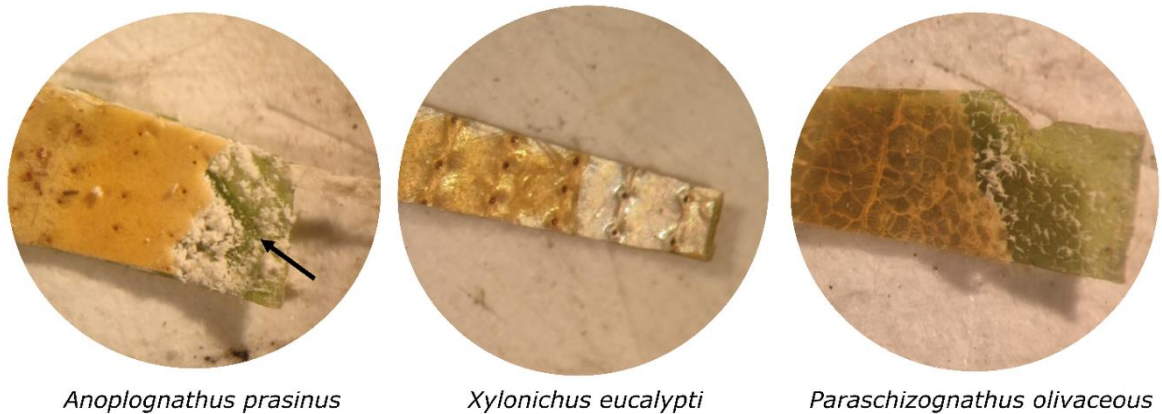


Figure B-1. Stereomicroscopy of the ventral side of the elytra

The yellow layer on the left of the photographs is the ventral lamination. The white underlay is exposed on the right side of the elytra. The differences in the white underlay between beetles are evident: in *A. prasinus* the white underlay is thick, dense and reflects the light diffusely. In *X. eucalypti* glossy reflections are visible by the naked eye. In *P. olivaceous* the white underlay is much more subtle and it seems to correspond to the honeycomb walls since the honeycomb array is visible through the lower lamination. The black arrow shows an example of the incomplete scraping of the white underlay. For our experiments we removed the yellow lower lamination, then we compared two samples: one with the intact composite material and one for which we completely scraped the white underlay to isolate the cuticle.

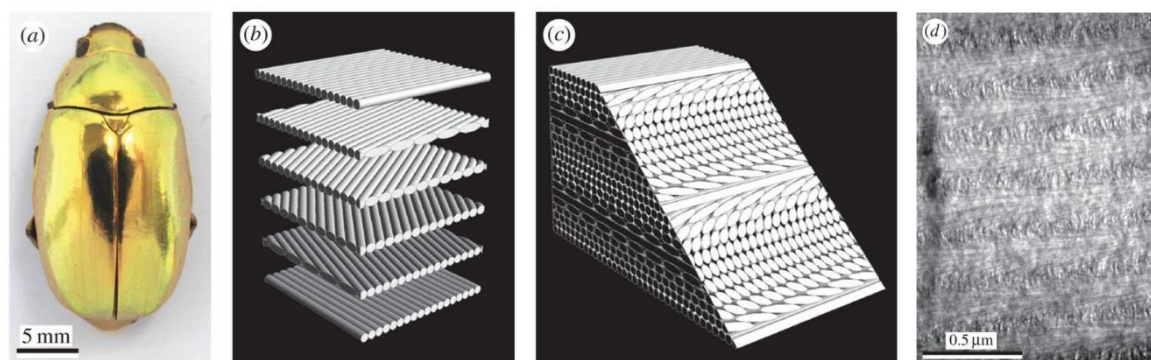


Figure B-2. Helicoidal chiral structures present in the cuticle of various scarab species

(a) Dorsal view of *C. resplendens*. (b) The colours in the scarab elytra are created by chitin fibres coiling in a helicoid array. (c) The oblique cut shows two Bouligand curves in the arrangement which consist of one pitch (one turn of the fibre). (d) TEM of an oblique section of the elytron of *C. resplendens* showing the Bouligand curves. Figure and legend modified from Finlayson E. D., McDonald L. T. and Vukusic P. (2017) Optically ambidextrous circularly polarized reflection from the chiral cuticle of the scarab beetle *Chrysina resplendens* J. R. Soc. Interface.

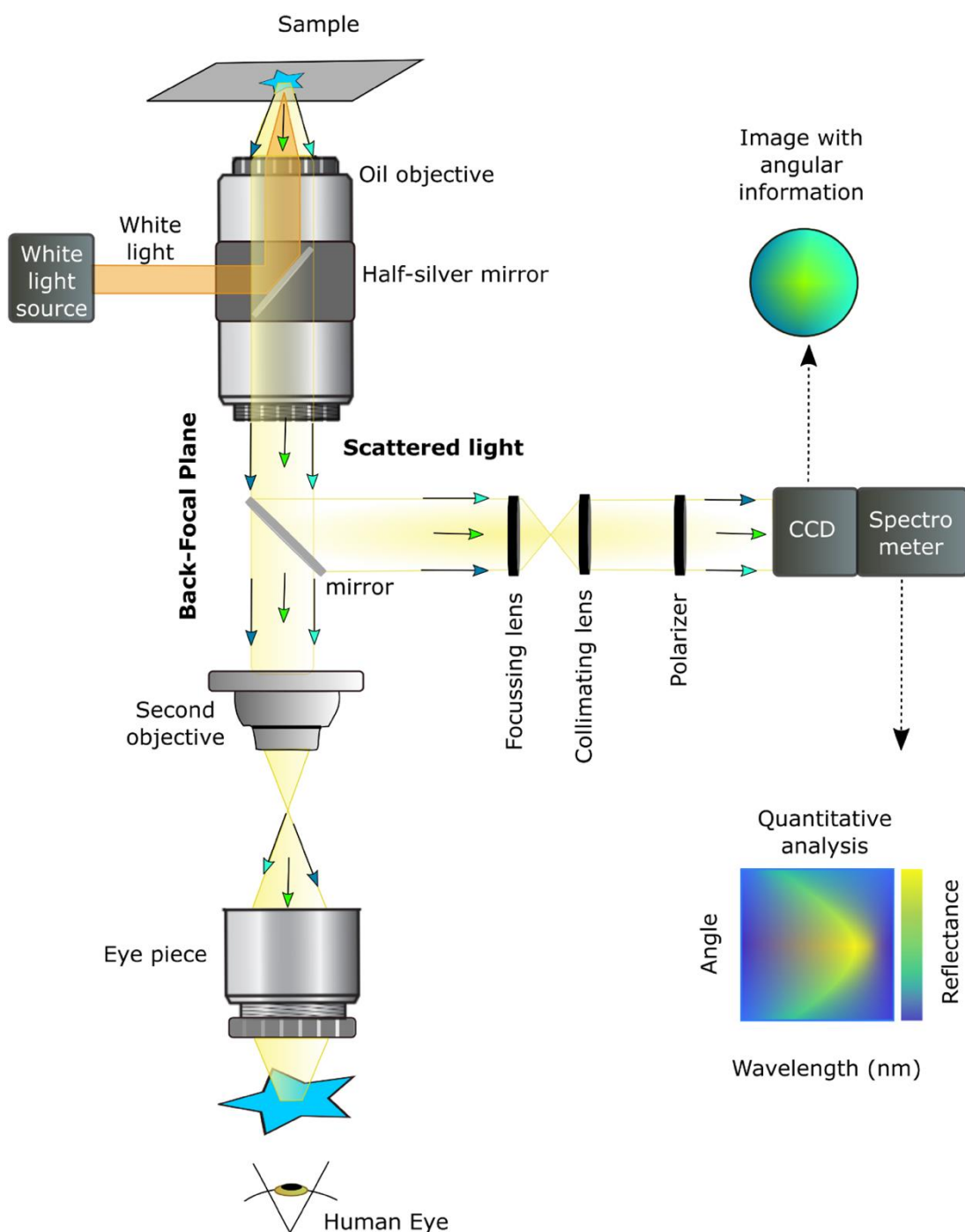


Figure B-3 Experimental set up for microscatterometry measurements

(See also article methods section). This technique consists in collecting and quantifying the light from the **back-focal plane** of a microscope set up (i.e. between two consecutive objectives before directing the beams to the eye piece) because in this section, the light rays travel parallel to each other, and the light scattering angle is transformed to spatial coordinate. Thus, light rays scattered normal to the sample surface will occur at the centre of the circular back-focal plane image, while light rays scattered at high angles will occur at the periphery. This facilitates the measurement of the amount of light per wavelength reflected by the sample at a given observation angle. The sample is illuminated with broadband unpolarized light through an oil immersion objective. The illumination light is expanded to fill the objective back aperture, thus exciting the sample with the full angular range as determined by the objective numerical aperture. The scattered light over the same angular range is collected by the objectives and an image of the back-focal plane is relayed to a spectrograph/CCD camera couple via a series of lenses and polarizer. This image can also be dispersed on one axis into

the different wavelengths, so that the resulting image quantifies reflectance (represented in the graph as a colour gradient) for each wavelength (x axis) reflected by the sample at a given angle (y axis). If the sample has multilayer-derived angle-dependent scattering, a characteristic half-moon shape is observed. If the sample is a diffuse scatterer, as in the case of the beetles studied in our experiment, a homogeneous pattern across the y axis is expected.

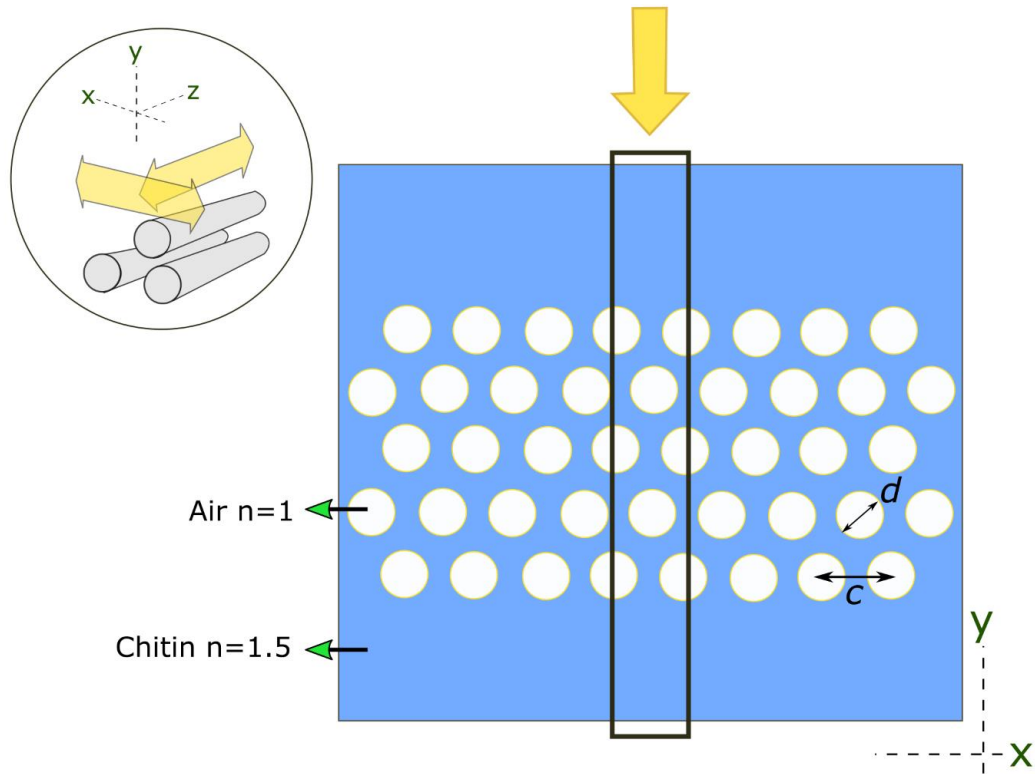


Figure B-4 Schematics of the photonic crystal model

The cylinders were modelled in a 2D structure as circles of air (refractive index $n = 1$) in a matrix of chitin (refractive index $n = 1.5$) in the $x - y$ plane with the light incident in the y direction (arrow). We considered the incoming light from the dorsal side of the elytron with the electric field parallel to the axis of the cylinders (y polarization) and perpendicular (x polarization) as shown in the inset. We also evaluated different combinations of cylinder diameter (d) and centre to centre distance (c).

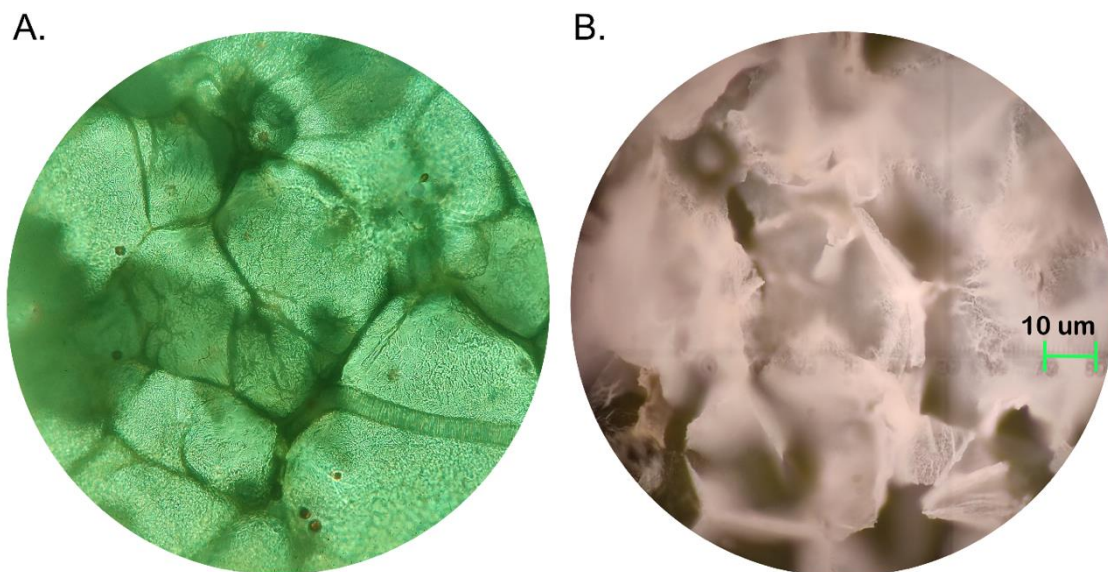


Figure B-5 Example of irregularities in the surfaces in *P. olivaceous* elytra

A) Surface of the cuticle in dorsal side of the elytra observed in transmitted light. **B)** Surface of the white underlay in the ventral side of the elytra observed in dark field. The spot diameter of the scatterometer beam was ~1 μm so we focused the beam on a relatively flat spot of these surfaces. However, the irregularities at different scales and directions make the scatterometry results less homogeneous than when performed in human-fabricated materials.

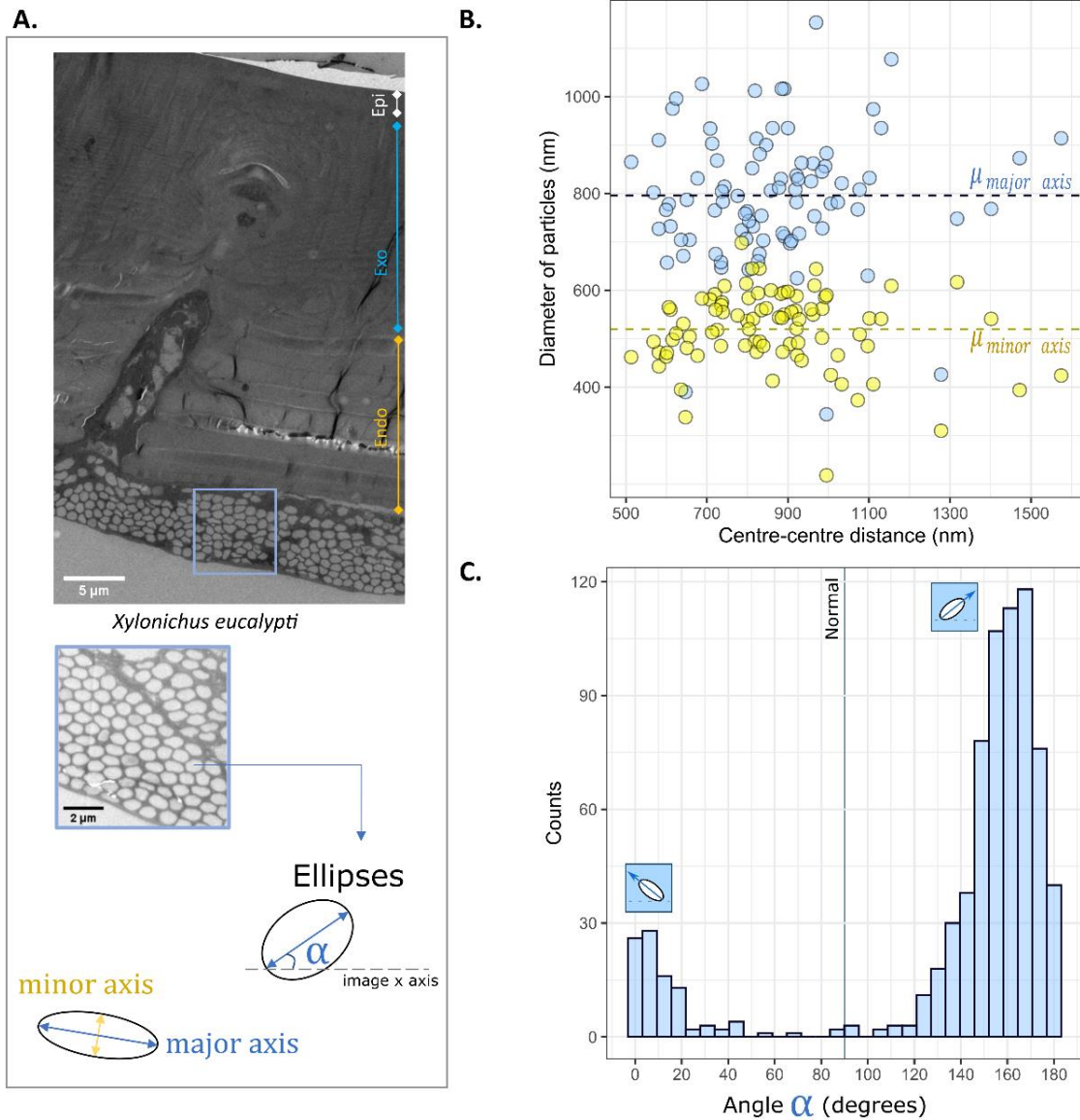


Figure B-6 Characterisation of the cylinders in the white underlay of *X. eucalypti*

A) TEM image of the composite material in the dorsal side of the cuticle of *X. eucalypti*. The transversal sections of the cylinders in the white underlay are elliptical. Thus, we calculated their major and minor axis as well as the angle between their major axis and the horizontal of the images (α). **B)** We did not find any correlation between the centre-to-centre distance between two adjacent ellipses and their size (major and minor axis) **C)** The major axis of the ellipses roughly aligns with the surface of the cuticle and the ventral surface of the elytra, but it varies between 0 and 30 degrees in the two orientations shown by the inset (symmetrical around the normal).

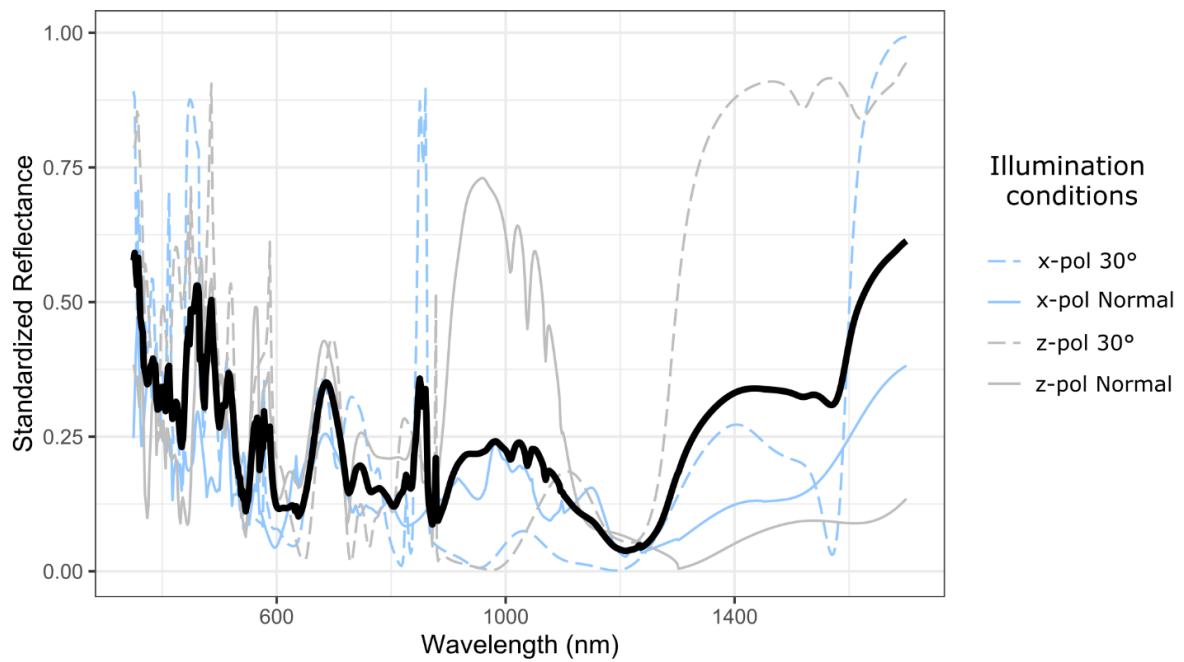


Figure B-7 Broadband reflectance in *X. eucalyptii* white underlay as a result of disorder

The black line represents the overall reflectance of the structure combining the 24 different scenarios modelled in our study. To obtain this trend we first averaged the reflectance at different spacing and particle size for each light condition - x and z polarization and Normal or 30 degrees angle of incidence (results plotted as indicated by the legend). Then, we averaged the resulting 4 reflectance spectra. This procedure ensures a weighted average considering that we did not model the same number scenarios for each angle of incidence. Higher reflectance could be obtained if more models with different particle size and separation were considered for the two light conditions. Nevertheless, it is clear that the natural structure under unpolarized light will exhibit a broadband reflectance across the whole spectrum, which explains its white appearance and high NIR reflectance.

Supplementary models

Methods

We ran numerical simulations of the structure using a custom MATLAB script adapted from the algorithm previously used to study quasi-coherent scattering from similar structures with two distinctive refractive indices producing diffuse blue scattering in birds (Prum & Torres, 2003b; Prum *et al.*, 1999b).

In this model we assumed that the air cylinders are of similar diameters but are not perfectly aligned (quasi-ordered matrix model). For this model we ran numerical simulations of the structure using a custom MATLAB script adapted from the algorithm previously used to study similar structures with two distinctive refractive indices producing diffuse blue scattering in birds (Prum & Torres, 2003b; Prum *et al.*, 1999b). In this case, we inputted TEM images of the white underlay with dark (electron-dense cuticle) and clear (hollow) regions that can be associated to two different indices of refraction, $n = 1.5$ (chitin) and $n = 1$ (air) respectively (Fig 6A). The algorithm performs two-dimensional Fast Fourier Transform (FFT) and radial averaging to convert the spatial information of the TEM images into a sinusoidal function known as Fourier Power Spectrum (FPS). A ring-like shape in the FPS (Fig S5) indicates repetitive patterns in the structure, therefore coherent scattering. Afterwards, a derivation of the Bragg equation is applied to describe the interaction between infinitely long cylinders and light approaching with its electric field in the plane perpendicular to the direction of the cylinders (cylinders modelled as delta functions) (Benedek, 1971). This calculates the relative power for the concentric rings in the FPS, i.e. the probability of each wavelength being reflected by the structure. We repeated this procedure for all 45 images TEM images of the white underlay in *X. eucalypti* and then averaged the results.

Results

The output of this model is the probability of each wavelength of being reflected by the structure. For *X. eucalyptii* we found that longer wavelengths have a higher probability of being reflected (**Figure B-8**). However, this algorithm only considers the x polarisation, i.e. the one perpendicular to the axis of the cylinders. This could explain the overall low probability of reflectance and also the absence of reflectance in the visible spectrum.

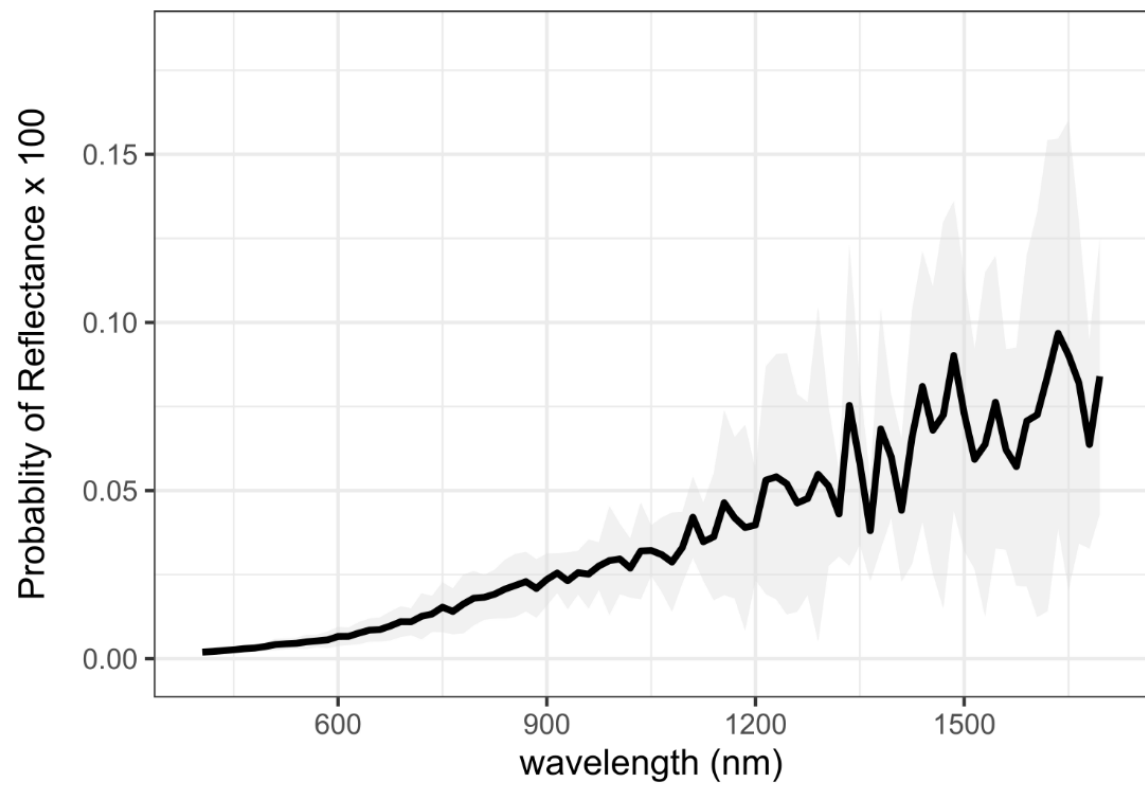


Figure B-8 Averaged results for our simulations with the quasi-ordered model

The shaded line represents the standard error around the average for each wavelength.

Appendix to Chapter 4

Can manipulation of visible and near-infrared
sunlight prevent overheating?

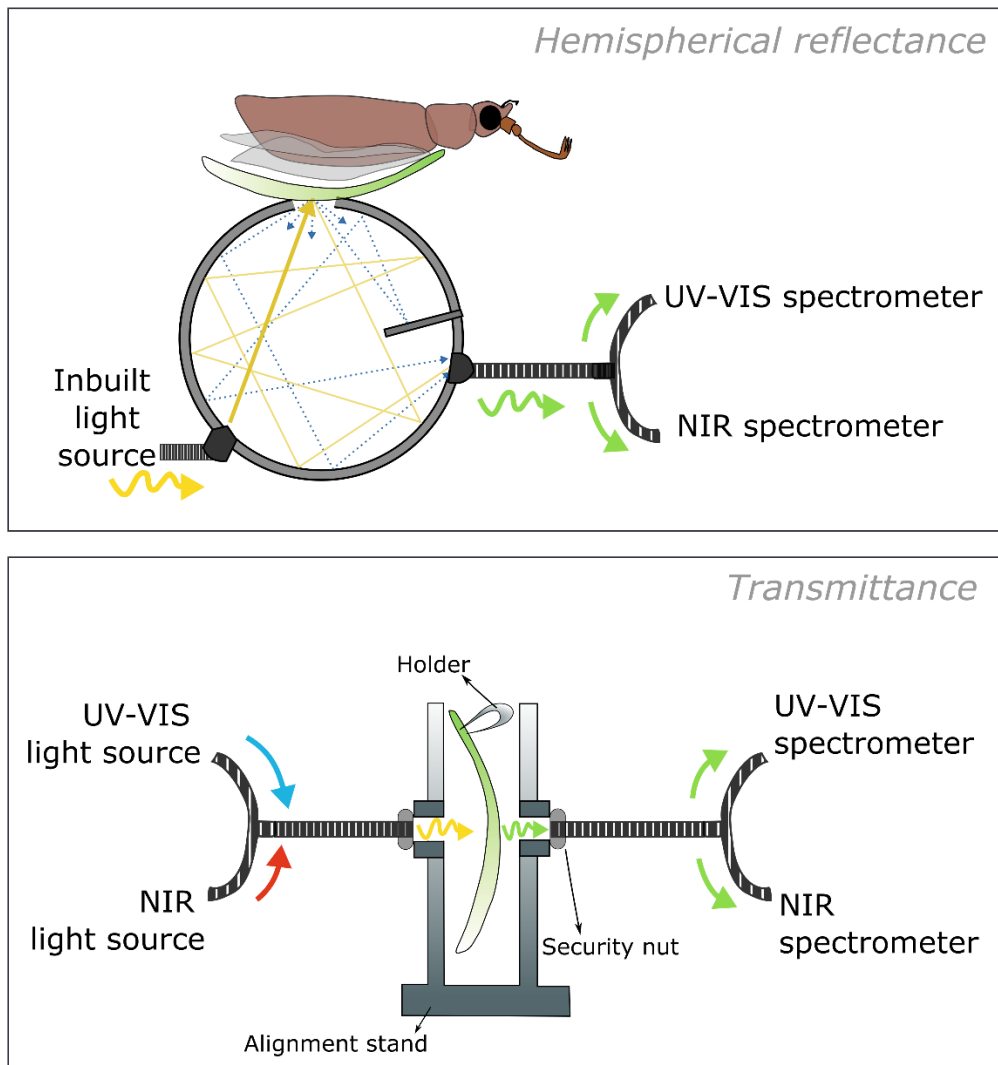


Figure C-1. Setup to measure the optical properties

We measured hemispherical reflectance (reflectance integrated across all angles) on the elytra still attached to the beetle body with an integrated sphere. The sample is illuminated with a broadband source (400 - 2100 nm) and the reflected light is collected by a bifurcated fibre that connects to two spectrometers UV-VIS (400-1000 nm) and NIR (1000-2100 nm). We measured transmittance in one elytron from each beetle specimen. To quantify the amount of light that can pass through it is crucial to ensure proper alignment. Thus, we used a metallic stand with two plaques with opposite pinholes. A security nut fixes the light and spectrometer fibre optics to the stand in the optimal position. We carefully suspended the elytron between the two plaques with a custom-made holder.

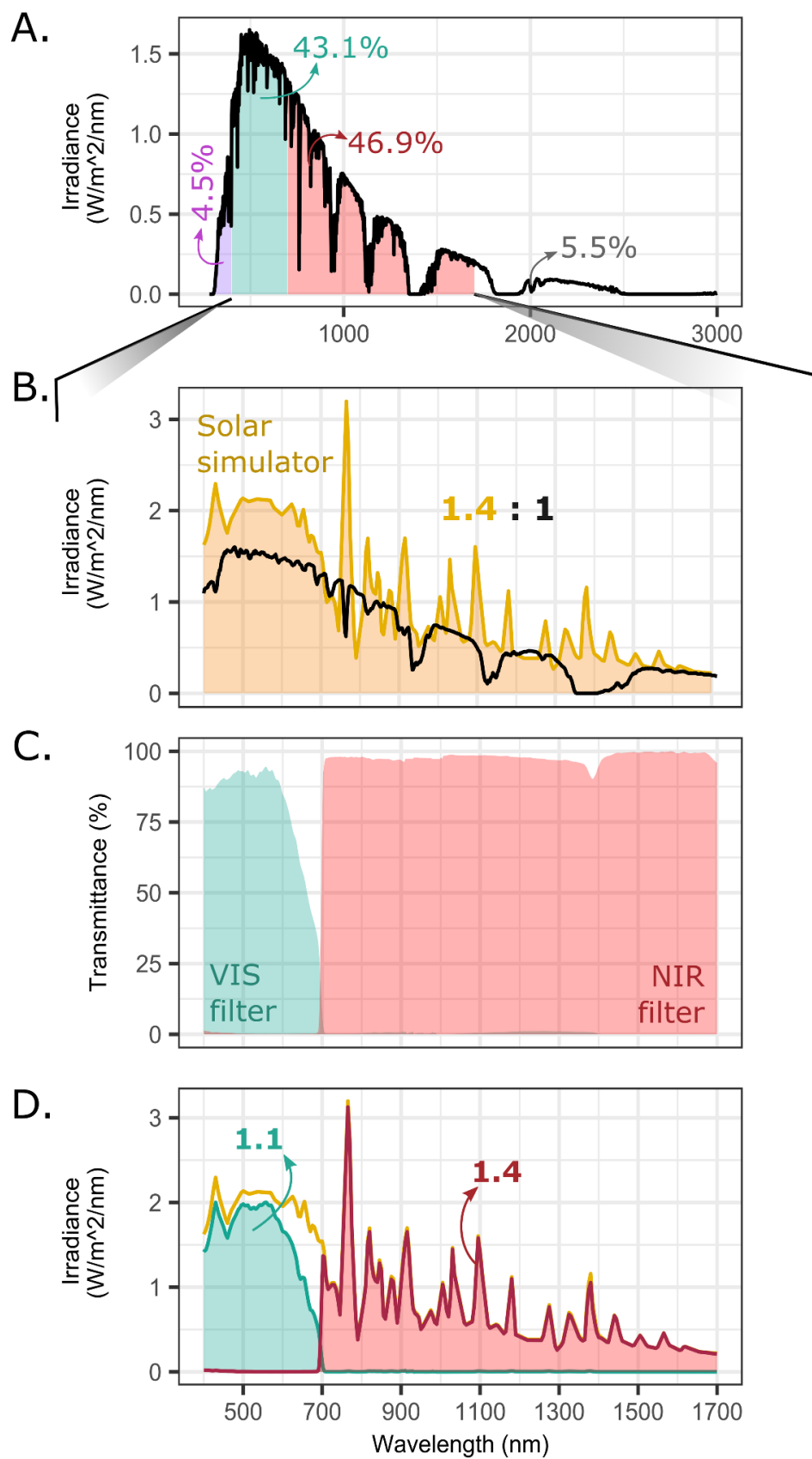


Figure C-2. Irradiance spectrum of solar simulator and filters

A) Irradiance spectrum of the sun and the percentage of energy associated to each spectral band UV, visible and NIR. The UV (wavelengths shorter than 300nm), and in the NIR beyond 1700 nm (not shaded) were not considered in our experiment, but together they only represent 10% of the energy available from the sun. **B)** Comparison between the spectrum of the solar simulator at 0.5 Sun (shaded area) and the solar (black lines) irradiance ($\text{W/m}^2 \text{ nm}$) in the wavelength range relevant for our experiment 400 to 1700 nm. Although the solar simulator has higher irradiance in the VIS region than the sun, the overall shape is similar. The numbers indicate the ratio solar simulator: solar irradiance energy in this wavelength range. **C)** Transmittance of VIS and NIR filters used in our experiment. These filters consisted in the following combinations of optical filters manufactured by Edmund Optics (Singapore): items #84727 and #49095 [KG-5 heat absorbing glass] were used for the VIS transmitting filter and items #84760 and #64703 were used for the NIR transmitting filter. **D)** Proportion of the solar simulator irradiance effectively reaching the platform inside the chamber after passing through each of the filters (shaded), in comparison to the original irradiance of the solar simulator (black line). The numbers indicate the ratio of effective incident light compared to the solar irradiance energy for each of the two wavelength ranges visible and NIR. The fact that the ratio is close to 1 in the three intervals used in our experiments (VIS, NIR and total) indicates that amount of light incident over our sample is similar to that expected under the sun.

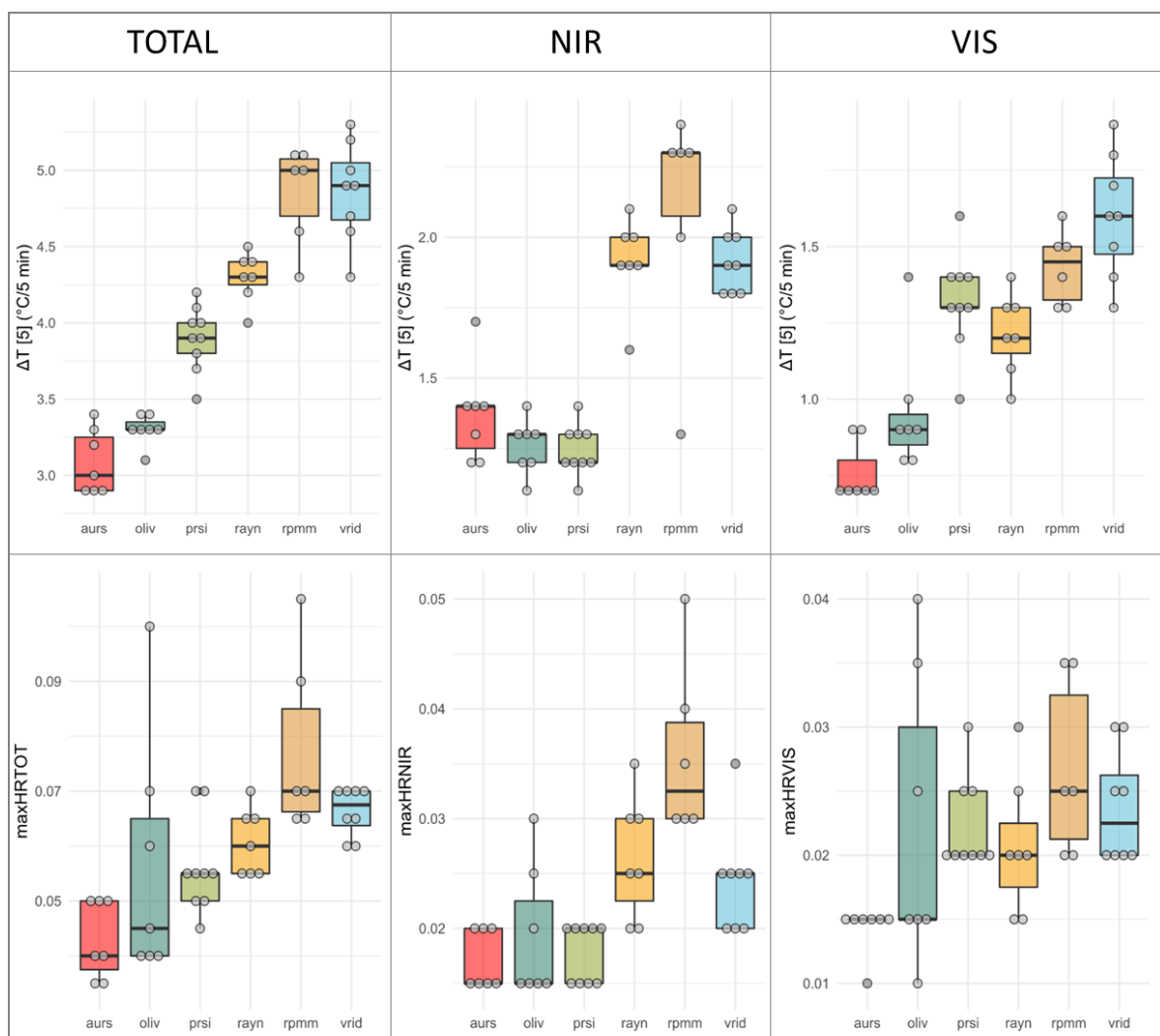


Figure C-3. Visualisation of raw values for repeatability

Boxplots showing the raw values of heating measurements for the 7 species used to test repeatability in the different spectral bands

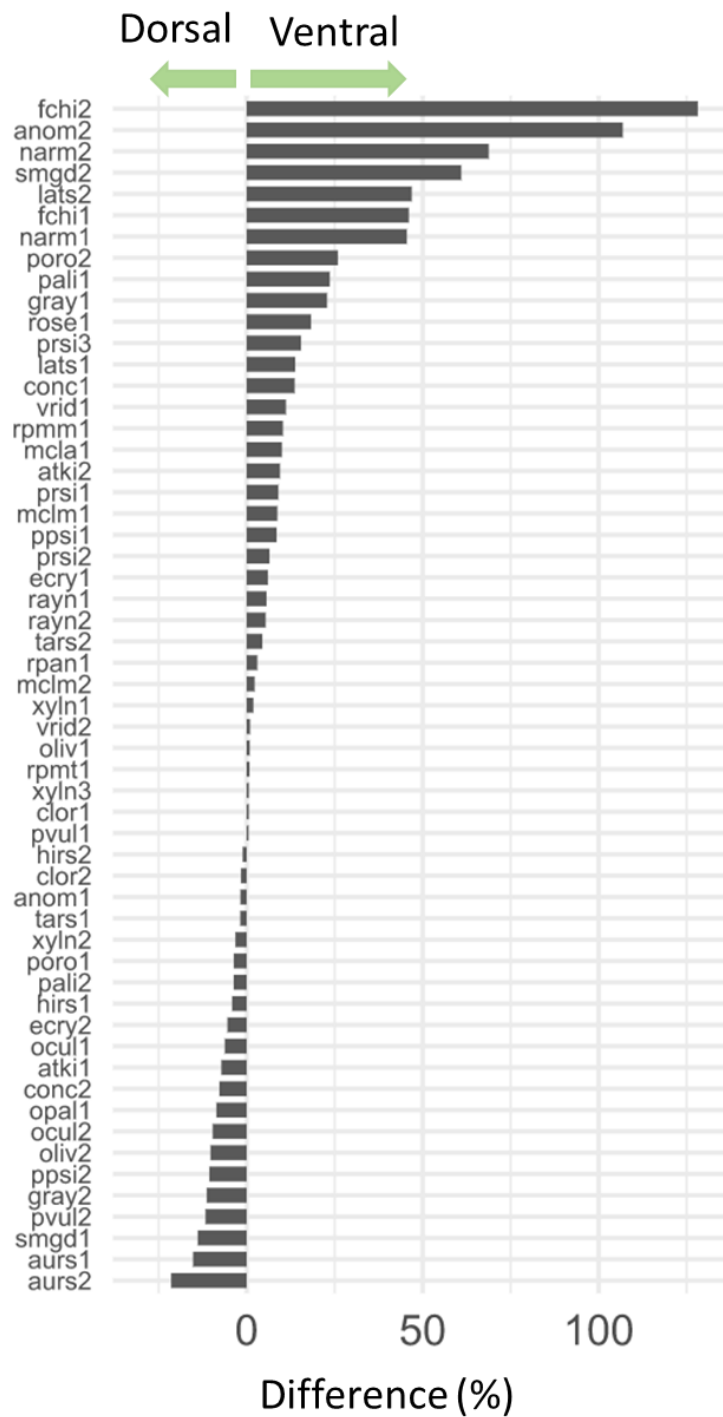


Figure C-4. Differences in transmittance on opposite sides of the elytra.

The difference between dorsal and ventral transmittance, was standardized as a percentage of the dorsal transmittance under total illumination. There is a subtle trend indicating higher transmittance from the ventral side of the elytra.

Supplementary tables

Table C-1. Specimens used in this experiment

List of species and ANIC reference number of the specimens used in this study

Code	Species	Lat	Long	ANIC Number
anom1	<i>Anomala antiqua</i>	-12.352	130.876	ANIC057768
anom2	<i>Anomala antiqua</i>	-19.233	146.09	NA
atki1	<i>Calloodes atkinsonii</i>	-16.47	145.22	NA
atki2	<i>Calloodes atkinsonii</i>	-15.03	145.09	NA
aur1	<i>Anoplognathus aureus</i>	-16.883	145.683	ANIC000260
aur2	<i>Anoplognathus aureus</i>	-16.883	145.683	ANIC000261
clor1	<i>Anoplognathus chloropyrus</i>	-30.6	151.55	ANIC000962
conc1	<i>Anoplognathus concolor</i>	-28.333	153.333	ANIC002611
conc2	<i>Anoplognathus concolor</i>	-32.167	151.5	ANIC002612
ecry1	<i>Epichrysus lamprimoides</i>	-34.96	117.86	NA
ecry2	<i>Epichrysus lamprimoides</i>	-34.96	117.86	NA
fchi1	<i>Calloodes frenchi</i>	-11.39	142.27	NA
fchi2	<i>Calloodes frenchi</i>	-11.39	142.27	NA
gray1	<i>Calloodes grayianus</i>	-18.96	144.987	NA
gray2	<i>Calloodes grayianus</i>	-17.69	123.6	NA
hirs1	<i>Anoplognathus hirsutus</i>	-30.4	152.35	ANIC002916
hirs2	<i>Anoplognathus hirsutus</i>	-34.583	149.567	ANIC002917
lats1	<i>Anoplostethus laetus</i>	-17.03	145.46	NA
lats2	<i>Anoplostethus laetus</i>	-17.81	145.59	NA
mcla1	<i>Anoplognathus macleayi</i>	-22.117	133.417	ANIC005833
mclm1	<i>Anoplognathus macleayi</i>	-23.017	132.7	ANIC003250
mclm2	<i>Anoplognathus macleayi</i>	-23.633	133.867	ANIC003251
narm1	<i>Anoplognathus narmarus</i>	-32.418	137.978	ANIC005462
narm2	<i>Anoplognathus narmarus</i>	-32.418	137.978	ANIC005463
ocul2	<i>Paraschizognathus ocularis</i>	-29.28	152.21	NA
oliv1	<i>Paraschizognathus olivaceous</i>	-31.743	152.563	NA
oliv2	<i>Paraschizognathus olivaceous</i>	-30.23	153.02	NA
opal1	<i>Anoplostethus opalinus</i>	-31.75	116.09	NA
pali1	<i>Anoplognathus pallidicollis</i>	-35.267	149.1	ANIC006061
pali2	<i>Anoplognathus pallidicollis</i>	-35.273	149.098	ANIC006062
poro1	<i>Anoplognathus porosus</i>	-16.917	145.767	ANIC009199
poro2	<i>Anoplognathus porosus</i>	-19.633	147.5	ANIC009200

Code	Species	Lat	Long	ANIC Number
ppsi1	<i>Paraschizognathus prasinus</i>	-33.837	150.413	NA
ppsi2	<i>Paraschizognathus prasinus</i>	-33.421	150.309	NA
prsi1	<i>Anoplognathus prasinus</i>	-30.383	152.983	ANIC010040
prsi2	<i>Anoplognathus prasinus</i>	-28.817	153.267	ANIC010045
prsi3	<i>Anoplognathus prasinus</i>	-28.2	153.183	ANIC010043
pvul1	<i>Anoplognathus parvulus</i>	-19.6	146.833	ANIC008650
pvul2	<i>Anoplognathus parvulus</i>	-19.517	147.1	ANIC008651
rayn1	<i>Calloodes rayneri</i>	-28.04	145.41	NA
rayn2	<i>Calloodes rayneri</i>	-28.04	145.41	NA
rose1	<i>Anoplostethus roseus</i>	-11.015	136.748	NA
rpan1	<i>Repsimus aeneus</i>	-26.56	153.08	NA
rpmm1	<i>Repsimus manicatus</i>	-33.546	150.895	NA
smgd1	<i>Anoplognathus smaragdinus</i>	-17.533	146.017	ANIC010862
smgd2	<i>Anoplognathus smaragdinus</i>	-16.917	145.767	ANIC010863
tars1	<i>Anoplognathus viriditarsis</i>	-30.983	151.583	ANIC011535
tars2	<i>Anoplognathus viriditarsis</i>	-34.967	149.033	ANIC011492
xyln1	<i>Xylonichus eucalypti</i>	-31.45	152.74	NA
xyln2	<i>Xylonichus eucalypti</i>	-30.52	152.38	NA
xyln3	<i>Xylonichus eucalypti</i>	-33.725	151.203	NA

Table C-2. Repeatability (R) of heating measurements

Heating Measurement	Spectral Range	R	Std Error	Deviance	p-value
ΔT_5 (°C/5 min)	WIDE	0.91	0.101	72.3	< 0.001
	NIR	0.79	0.139	43.5	< 0.001
	VIS	0.78	0.158	41.4	< 0.001
$maxHR$ (max. $\Delta^\circ C / sec$)	WIDE	0.42	0.187	9.04	0.0013
	NIR	0.64	0.188	22.1	< 0.001
	VIS	0.22	0.145	2.42	0.0599

Table C-3. Reflectivity as a predictor of heating.

Results of 6 models testing how heating (ΔT_5 and maxHR) is predicted by reflectivity and size (elytra length)

Response variable	Spectral Range	R2 (%)	Parameter	Partial R2 (%)	Estimate	Std Error	p-value
ΔT_5 (°C/5 min)	Total	38.76	Intercept	NA	3.778	0.353	< 0.001
			Reflectivity	21.11	-0.026	0.006	< 0.001
			Size	16.62	0.516	0.141	< 0.001
	NIR	28.43	Intercept	NA	1.565	0.211	< 0.001
			Reflectivity	19.67	-0.010	0.002	< 0.001
			Size	8.61	0.206	0.083	< 0.05
	VIS	38.17	Intercept	NA	0.879	0.134	< 0.001
			Reflectivity	5.20	-0.006	0.003	< 0.05
			Size	24.79	0.241	0.055	< 0.001
maxHR (max. Δ °C/ sec)	Total	31.73	Intercept	NA	0.0724	0.0112	< 0.001
			Reflectivity	20.99	-0.0007	0.0002	< 0.001
			Size	6.53	0.0092	0.0045	< 0.05
	NIR	37.77	Intercept	NA	0.0372	0.0043	< 0.001
			Reflectivity	35.88	-0.0003	0.0001	< 0.001
			Size	0.002	-0.0003	0.0017	0.877
	VIS	21.31	Intercept	NA	0.0267	0.0054	< 0.001
			Reflectivity	8.78	-0.0003	0.0001	< 0.05
			Size	1.06	0.0014	0.0022	0.532

Note: The three spectral bands were TOTAL (400 - 1700 nm), NIR (700 - 1700 nm) and VIS (400 - 700 nm). Units of the estimates and standard errors are specified in the response variable column. The reported estimates already account for the effect of the side of the platform in which the samples were placed. The difference between the overall R^2 and the sum of the partial R^2 corresponds to the variance (%) explained by the side. Its effect was significant ($p < 0.05$) only for ΔT_5 and maxHR in VIS.

Table C-4. Transmissivity as a predictor of heating

Results of 6 models testing how the two response variables (ΔT_5 and maxHR) are predicted by transmissivity and size (elytra length)

Response variable	Spectral Range	R2 (%)	Parameter	Partial R2 (%)	Estimate	Std Error	p-value
ΔT_5 (°C/5 min)	Total	21.74	Intercept	NA	3.713	0.446	< 0.001
			Transmissivity	11.61	-0.013	0.007	0.045
			Size	6.02	0.310	0.168	0.071
	NIR	7.24	Intercept	NA	1.330	0.272	< 0.001
			Transmissivity	1.97	-0.001	0.003	0.605
			Size	3.81	0.139	0.101	0.176
	VIS	38.52	Intercept	NA	0.926	0.139	< 0.001
			Transmissivity	9.68	-0.009	0.004	0.037
			Size	18.34	0.208	0.056	< 0.001
maxHR (max. $\Delta^\circ\text{C}/\text{sec}$)	Total	12.19	Intercept	NA	0.0602	0.0141	< 0.001
			Transmissivity	1.03	-0.0001	0.0002	0.649
			Size	2.96	0.0060	0.0053	0.267
	NIR	6.13	Intercept	NA	0.0279	0.0060	< 0.001
			Transmissivity	0.68	0.0000	0.0001	0.786
			Size	0.31	0.0013	0.0022	0.567
	VIS	17.33	Intercept	NA	0.0269	0.0058	< 0.001
			Transmissivity	2.76	-0.0002	0.0002	0.183
			Size	0.26	0.0004	0.0023	0.857

Appendix to Chapter 5

Christmas beetles' reflectivity, absorptivity and polarisation do not follow one universal ecogeographical rule

Note: In our manuscript we used PGLS models for most of the statistical analysis. For each model we checked if the assumptions were met. To see further details on this, please check the HTML version of our code.

Supplementary figures

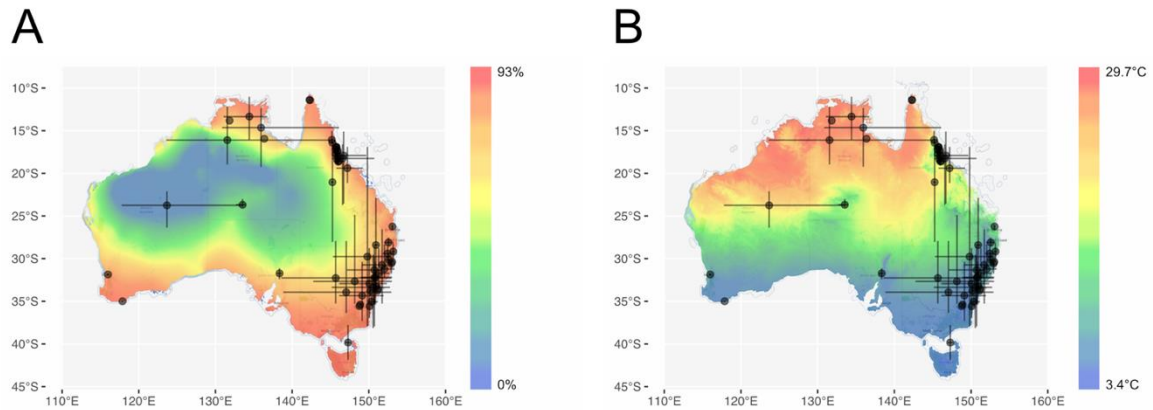


Figure D-1 Christmas beetles' distribution

The dots represent the mean latitude and longitude for the location of each species studied in this work. The heatmap in A) shows the annual mean relative humidity and in B) the annual mean temperature included here only for visualization. Species of Christmas beetles experience a wide range of climatic conditions.

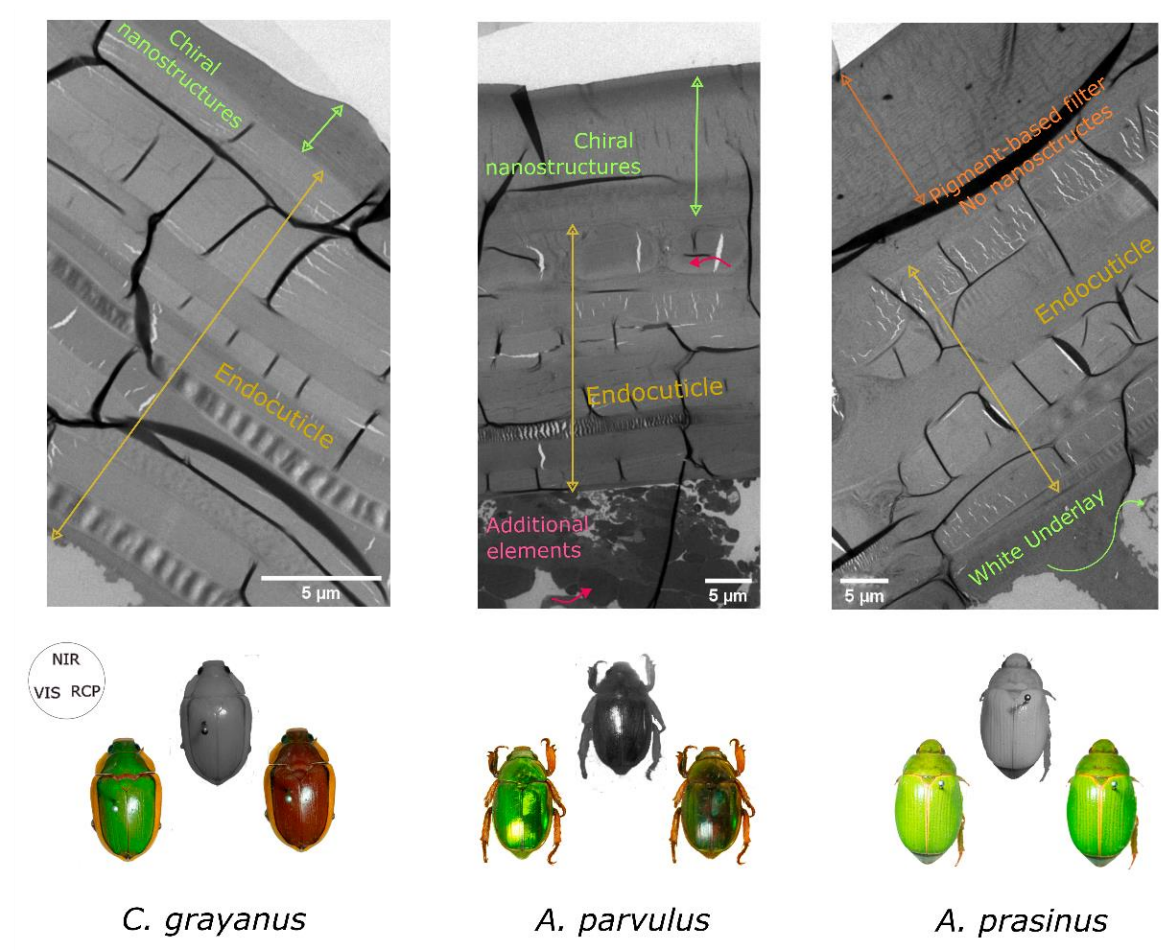


Figure D-2 Different mechanisms in the cuticles of Christmas beetles

Top row: Transversal TEM sections stained with heavy metals for block face imaging. Structures in *A. prasinus* named based on (Ospina-Rozo *et al.*, 2022a). Bottom row: pictures of the corresponding species under the visible, near-infrared and right circularly polarised (RCP) filters. A considerable change in colour under the RCP filter is evidence of polarisation, a landmark of chiral structures. In *C. grayanus*, a very thin (~2.5µm) simple chiral array produces polarised reflections of a saturated green. In *A. parvulus*, a large chiral array (~14µm) combined with additional elements produces polarised reflections of a broad-band greenish gold. These two beetles were also studied in (Parker *et al.*, 1998). In *A. prasinus* the non-chiral composite material produces non-polarised green.

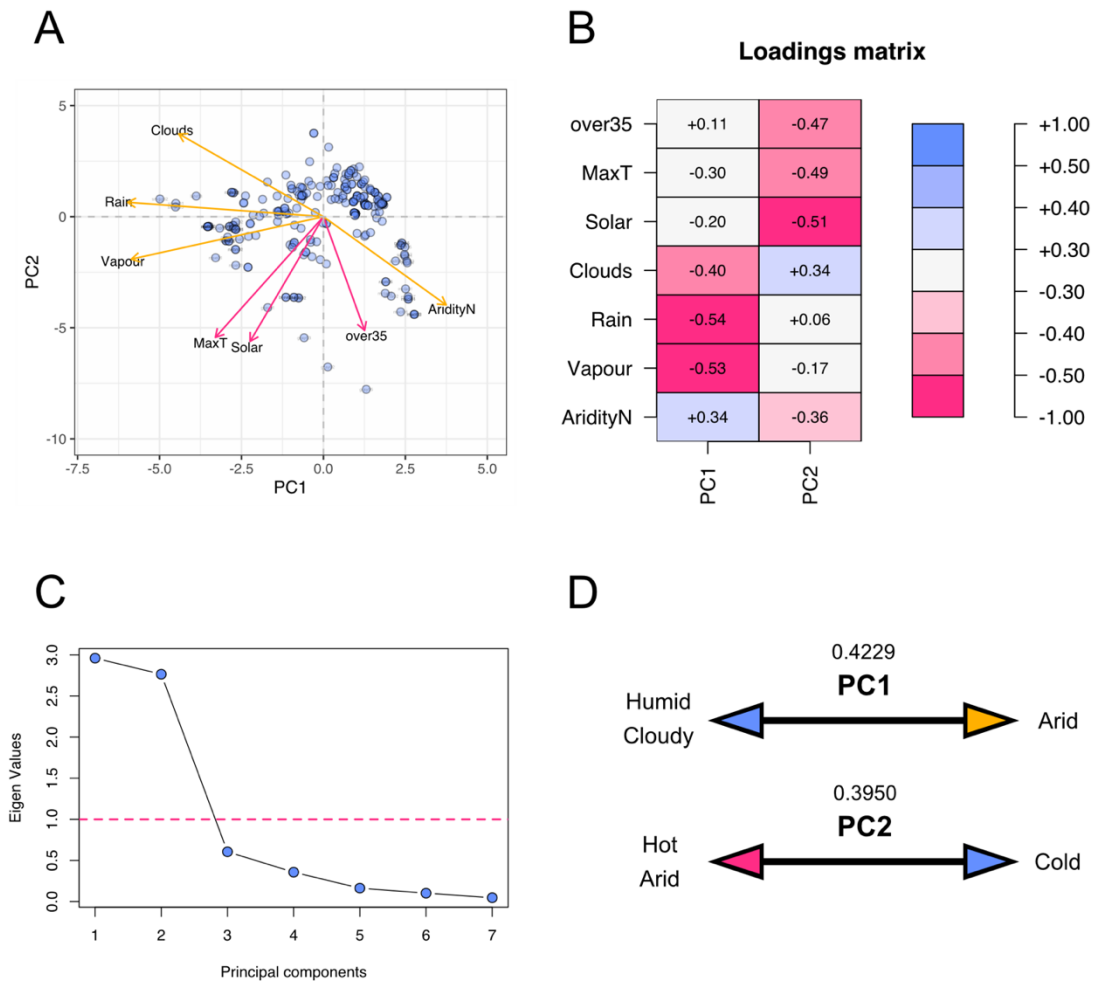


Figure D-4 Details of the PCA analysis used to summarise the ecological variables into two components

A) graphical representation of the variation in PC1 and PC2 values for all individuals and the directions of the variables. **B)** Loadings matrix. Correlations between ecological variables and the first 2 principal components. Scale bar: Significant correlations (beyond -0.3 to +0.3 range) are shaded in colours. PC1 correlates negatively with clouds, rain and water vapor. PC2 correlates negatively with the maximal temperature, the number of days with temperature over 35, solar radiation and aridity. **C)** We only considered the first two components since they have eigenvalues above 1. **D)** All the ecological variables can be summarised in PC1 - humidity and PC2 - temperature. These two components explain a total of 82% of the variation. PC1 explains 42.3% and PC2 explains 39% of the variation.

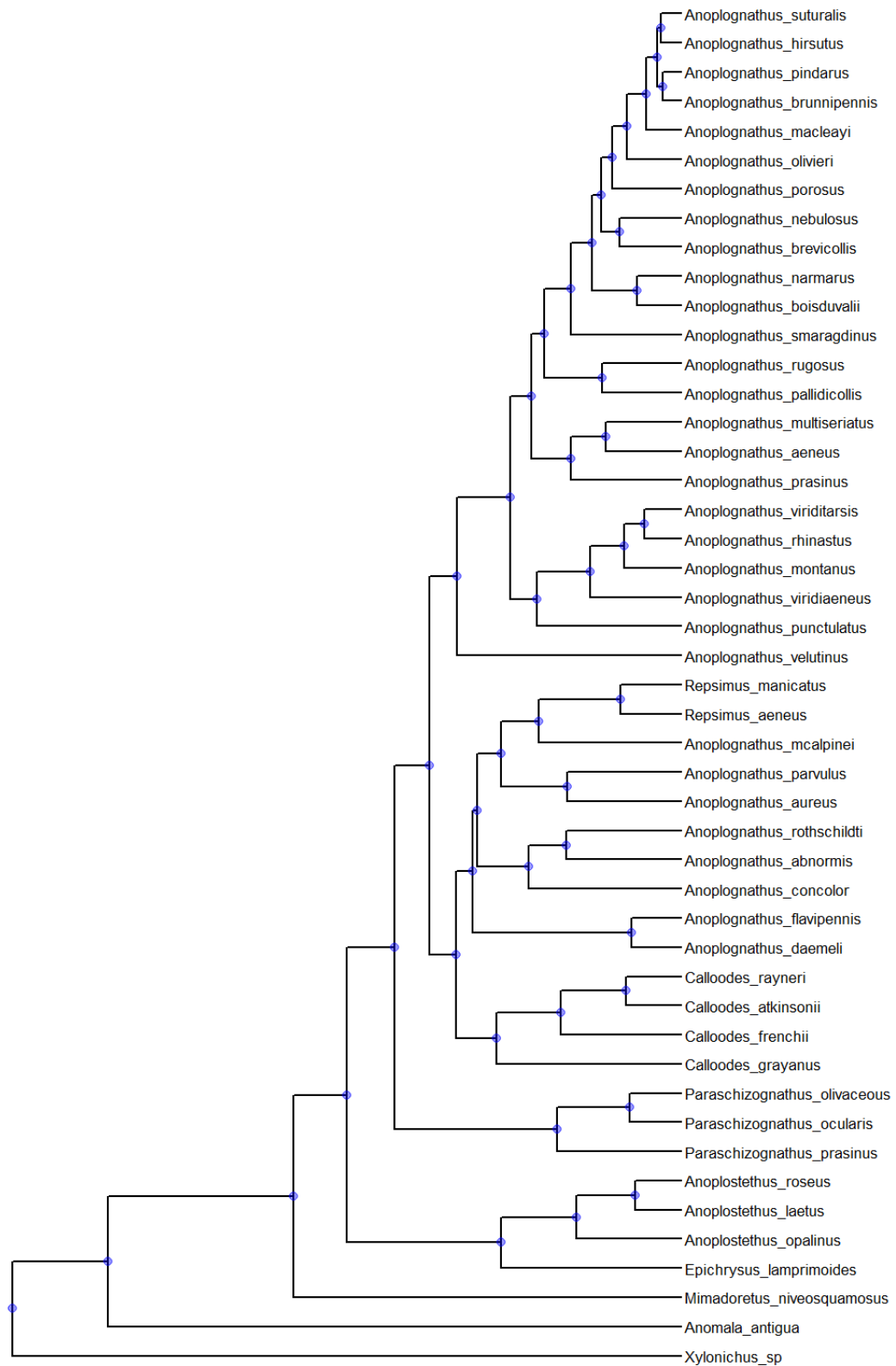


Figure D-5 Phylogenetic tree

This tree was built using the COI data of Mitchell (2015) and additional framework and data from Gunter et al. (2016).

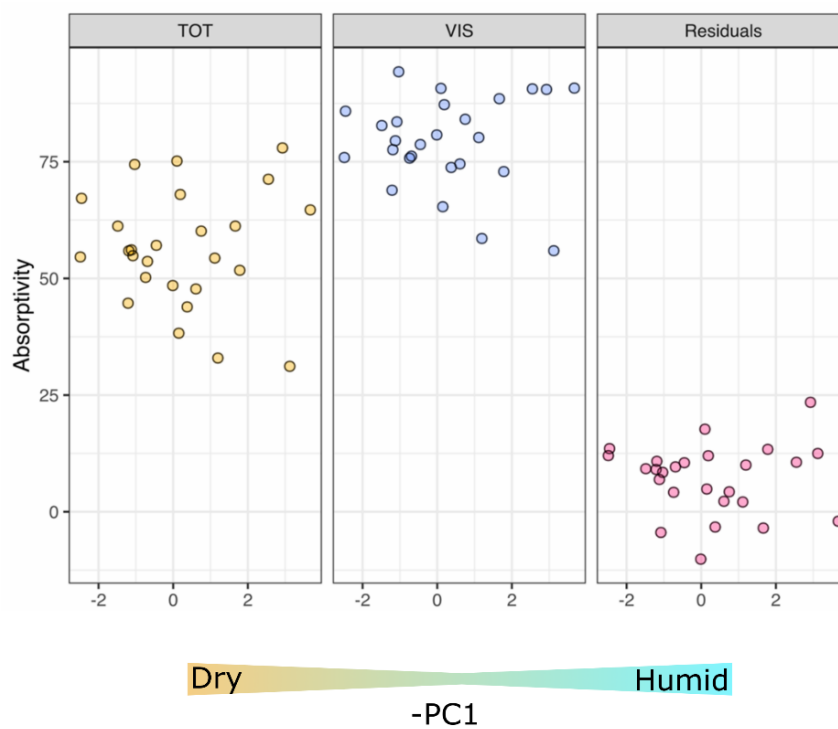


Figure D-6 Humidity variables are not a good predictor of elytral absorptivity

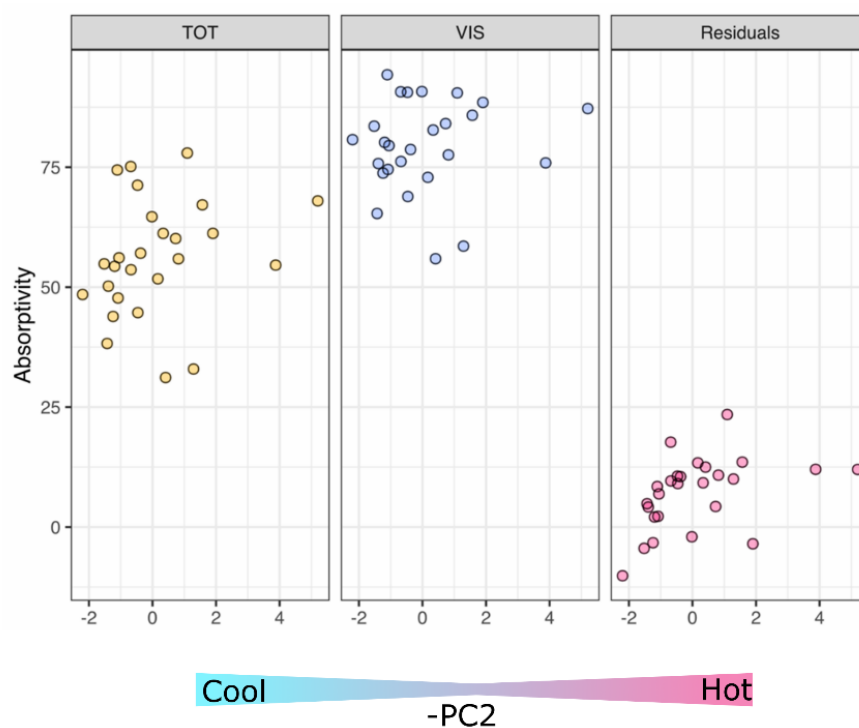


Figure D-7 Temperature variables are not a good predictor of elytral absorptivity

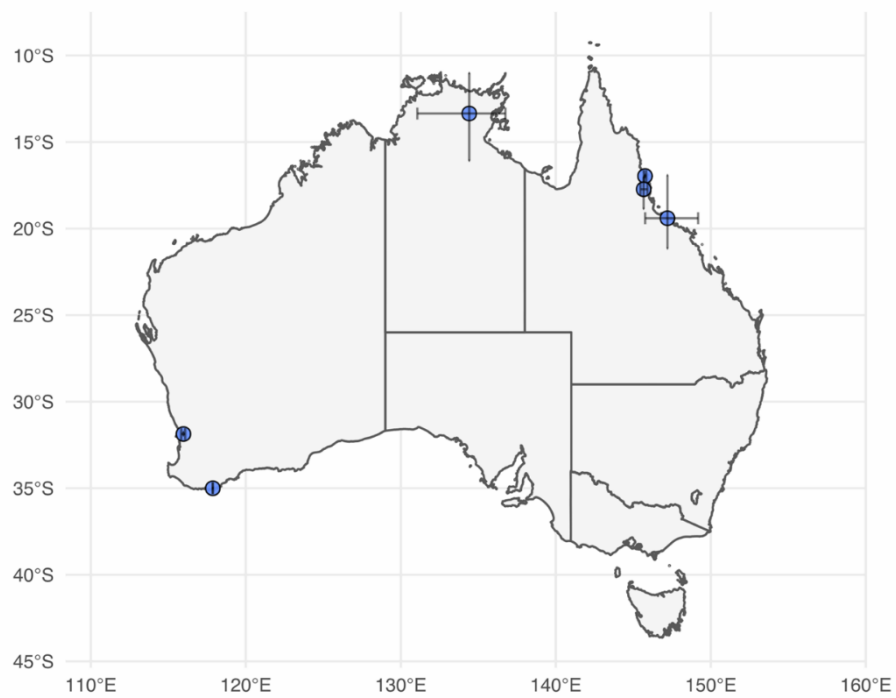


Figure D-8 Locations of the species that cluster due to their unusually high NIR and visible reflectivity

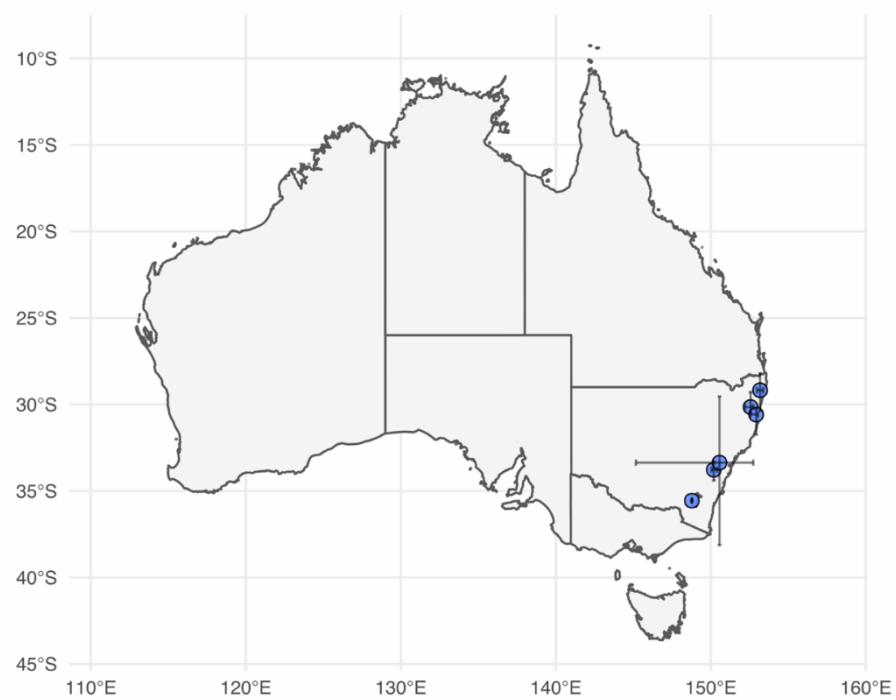


Figure D-9 Locations of the species that have enhanced NIR reflectivity due to a white underlay

Supplementary tables

Table D-1. Specimens used in this experiment

The detailed list of the ANIC museum specimens used in this research can be downloaded here: [Table1_ExcelFormat](#) and an interactive version can also be found in our [HTML code](#).

Table D-2. Pairwise Pearson's correlation coefficient between ecological variables

We remove variables with correlations >0.8. The three vegetation variables are related to water vapour and rain so they were not considered in the PCA analysis. Minimum and maximum temperature are also correlated so we used only the latter.

	C3Macro	C3Meso	C4Mega	over35	MaxT	MinT	Solar	Clouds	Rain	Vapour	AridityN
C3Macro	1.00	0.57	0.99	-0.09	0.68	0.87	0.47	0.55	0.84	0.95	-0.36
C3Meso	0.57	1.00	0.48	-0.47	-0.03	0.26	-0.23	0.73	0.64	0.42	-0.61
C4Mega	0.99	0.48	1.00	-0.02	0.73	0.90	0.53	0.49	0.81	0.96	-0.32
over35	-0.09	-0.47	-0.02	1.00	0.49	0.22	0.47	-0.55	-0.20	-0.01	0.40
MaxT	0.68	-0.03	0.73	0.49	1.00	0.91	0.83	-0.10	0.38	0.73	0.23
MinT	0.87	0.26	0.90	0.22	0.91	1.00	0.72	0.21	0.64	0.92	-0.06
Solar	0.47	-0.23	0.53	0.47	0.83	0.72	1.00	-0.26	0.23	0.53	0.28
Clouds	0.55	0.73	0.49	-0.55	-0.10	0.21	-0.26	1.00	0.61	0.52	-0.59
Rain	0.84	0.64	0.81	-0.20	0.38	0.64	0.23	0.61	1.00	0.77	-0.67
Vapour	0.95	0.42	0.96	-0.01	0.73	0.92	0.53	0.52	0.77	1.00	-0.27
AridityN	-0.36	-0.61	-0.32	0.40	0.23	-0.06	0.28	-0.59	-0.67	-0.27	1.00

Table D-3. Exact number of phylogenetic trees used on each model

Correlations	Model	Trees
Climate	TOT Reflectivity	997
	VIS Reflectivity	998
	NIR Reflectivity	997
	Residuals Reflectivity	999
	TOT Absorptivity	964
	VIS Absorptivity	965
	NIR Absorptivity	999
	Residuals Absorptivity	1000
	Polarisation	989
	Size	997
Correlations between variables	Degree Polarisation - VIS Reflectivity	980
	Degree Polarisation - NIR Reflectivity	1001
	Degree Polarisation - Residuals Reflectivity	1001
	Degree Polarisation - VIS Absorptivity	963
	Degree Polarisation - NIR Absorptivity	1001
	Degree Polarisation - Residuals Absorptivity	1001
	Right-Handed Pol RGB - VIS Reflectivity	992
	Right-Handed Pol RGB - NIR Reflectivity	1001
	Right-Handed Pol RGB - Residuals Reflectivity	1001
	Right-Handed Pol RGB - VIS Absorptivity	963
	Right-Handed Pol RGB - NIR Absorptivity	971
	Right-Handed Pol RGB - Residuals Absorptivity	1001

Table D-4. Phylogenetic signal determined by Pagel's lambda

Lambda values closer to 1 indicate high phylogenetic signal. P-values < 0.05 mean that lambda is significantly different from 0, i.e. close relatives are more similar than random pairs of species.

Variable	Lambda	P-value
Total reflectivity	0.8197	0.5718
Visible reflectivity	0.8826	0.1707
Nir reflectivity	0.8161	0.2247
NIR residuals reflectivity	0.9379	0.0053
Total absorptivity	0.9713	0.2105
Visible absorptivity	0.9647	0.3340
Nir absorptivity	0.9736	0.1386
NIR residuals absorptivity	0.8427	0.0206
Polarisation	0.9552	< 0.001

Table D-5. PGLS models for Reflectivity and absorptivity without outliers

Posterior 95% confidence intervals C.I. of the regression estimates, and p-values obtained by applying the PGLs function on >900 trees to account for phylogenetic uncertainty. The highlighted results provide evidence for correlations due to their estimate interval not overlapping with 0 and p values lower than 0.05. After excluding *A. prasinus* and the species belonging to the genus *Xylonichus* and *Paraschizognathus* from our analysis the correlations between optical properties and PC, and the interaction term PC2 x Size disappeared.

Optical property	Predictor	Estimate in MCC	Estimate interval	p-value
NIR reflectivity (Including VIS reflectivity as a predictor)	PC1	0.862	-0.170 ; 1.414	0.643 ; 1.000
	PC2	4.996	2.799 ; 6.543	0.0423 ; 0.413
	Size	0.661	-0.314 ; 2.034	0.476 ; 1.000
	PC1 x Size	-0.136	-0.379 ; 0.398	0.724 ; 1.000
	PC2 x Size	-2.050	-2.597 ; -1.196	0.065 ; 0.394
	Visible	0.969	0.930 ; 1.009	< 0.001 ; < 0.001
Residuals (NIR-VIS regression and corrected for phylogenetic relatedness)	PC1	1.398	0.533 ; 1.986	0.445 ; 0.837
	PC2	5.801	3.593 ; 7.236	0.027 ; 0.282
	Size	-0.088	-0.791 ; 1.097	0.690 ; 1.000
	PC1 x Size	-0.380	-0.621 ; 0.080	0.628 ; 1.000
	PC2 x Size	-2.355	-2.867 ; -1.507	0.0320 ; 0.257
NIR absorptivity (Including VIS reflectivity as a predictor)	PC1	-0.244	-0.836 ; 0.734	0.868 ; 1.000
	PC2	-5.924	-11.022 ; -0.962	0.172 ; 0.905
	Size	6.479	4.777 ; 8.122	0.180 ; 0.438
	PC1 x Size	0.062	-0.483 ; 0.387	0.851 ; 1.000
	PC2 x Size	2.112	0.203 ; 3.944	0.203 ; 0.949
	Visible	1.338	1.306 ; 1.351	< 0.001 ; < 0.001
Absorptivity Residuals (NIR-VIS regression and corrected for phylogenetic relatedness)	PC1	-0.317	-0.948 ; 0.531	0.850 ; 1.000
	PC2	-6.063	-11.048 ; -1.053	0.152 ; 0.892
	Size	6.519	4.798 ; 8.211	0.160 ; 0.420
	PC1 x Size	0.098	-0.385 ; 0.430	0.844 ; 1.000
	PC2 x Size	2.168	0.252 ; 4.016	0.181 ; 0.935

Table D-6. Polarisation correlations in the full data set and three subsets

This was done to evaluate if the correlations observed in our PGLs models are driven by outliers. The negative correlation between NIR reflectivity and polarisation is still present even after excluding the outliers. Absorptivity results are less robust probably due to the smaller data set.

Test	Band	Subset	df	Estimate	Interval	p-value	Conclusion
Polarisation - Reflectivity	VIS	All	45	0.224	-0.067 ; 0.48	0.13	No correlation
		No underlay	40	0.212	-0.098 ; 0.485	0.177	
		No cluster	39	-0.233	-0.505 ; 0.08	0.143	
		No special cases	34	-0.260	-0.542 ; 0.075	0.126	
	NIR	All	45	-0.251	-0.502 ; -0.502	0.088	Negative correlation Cluster spp seem to have higher NIR reflectivity than the rest
		No underlay	40	-0.154	-0.437 ; 0.158	0.331	
		No cluster	39	-0.518	-0.712 ; -0.251	0.001	
		No special cases	34	-0.514	-0.721 ; -0.223	0.001	
	Residuals	All	45	-0.537	-0.714 ; -0.295	<0.001	Negative correlation
		No underlay	40	-0.557	-0.736 ; -0.304	<0.001	
		No cluster	39	-0.527	-0.718 ; -0.262	<0.001	
		No special cases	34	-0.575	-0.76 ; -0.303	<0.001	
Polarisation - Absorptivity	VIS	All	24	0.008	-0.38 ; 0.394	0.967	Cluster spp seem to have lower VIS absorptivity than the rest
		No underlay	19	-0.003	-0.434 ; 0.429	0.99	
		No cluster	19	0.603	0.231 ; 0.821	0.004	
		No special cases	14	0.604	0.155 ; 0.846	0.013	
	NIR	All	24	0.214	-0.189 ; 0.556	0.293	Positive correlation Cluster spp seem to have lower NIR absorptivity than the rest
		No underlay	19	0.077	-0.367 ; 0.492	0.741	
		No cluster	19	0.642	0.292 ; 0.841	0.002	
		No special cases	14	0.584	0.125 ; 0.837	0.017	
	Residuals	All	24	0.419	0.038 ; 0.694	0.033	Correlation driven mainly by the two groups of special cases
		No underlay	19	0.209	-0.245 ; 0.587	0.364	
		No cluster	19	0.373	-0.07 ; 0.693	0.096	
		No special cases	14	0.179	-0.347 ; 0.62	0.506	

Note: “No underlay” excludes *A. prasinus*, *Xylonychus* and *Paraschizognathus* since data in Chapter 3 showed they have a different photonic mechanism. “No cluster” excludes *A. aureus*, *A. parvulus*, *A. roseus*, *A. laetus*, *A. opalinus* and *E. lamprimoides* since they deviate from the general trend in figure 5-5. “No special cases” is the intersection between the previous two sets.