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# Polymorphism of *Colias croceus* from the Azores caused by differential pterin expression in the wing scales

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ABSTRACT

The pierid butterfly *Colias croceus* (Geoffroy in Fourcroy, 1785), established in the Azores archipelago, is polymorphic with six forms, *C. croceus f. croceus* ♂ and ♀, *C. c. f. cremonae* ♂ and ♀, *C. c. f. helice* ♀, and *C. c. f. cremonaehelice* ♀. We investigated the optical mechanisms underlying the wing colouration of the butterflies by performing spectrophotometry and imaging scatterometry of the variously coloured wing areas and scales. The scale colouration is primarily due to wavelength-selective absorption of incident light by pterins expressed in granular beads in the wing scales, but thin film reflections of the scales' lower lamina and scale stacking also contribute. Three forms (*croceus* ♂ and ♀ and *helice* ♀) are consistent with the patterns of the well-known '*alba*' polymorphism. We postulate the coexistence of a second polymorphism, '*cremonae*', to understand the three other forms (*cremonae* ♂ and ♀, and *cremonaehelice* ♀), which are characterized by the absence of red pigment, presumably due to the differential blocking of erythropterin expression.

## 1. Introduction

Polymorphism is a widespread phenomenon among butterflies, which is usually due to the variable pigmentation of the wing scales. In Coliadinae, the well-known sex-limited '*alba*' polymorphism (white and orange females) is ancestral and ubiquitous, and concerns 55% of the actually described *Colias* species. The polymorphism is maintained by different selective pressure balances (Limeri and Morehouse, 2016; Limeri, 2017). The advantages of the '*alba*' white morph is based on developmental benefits, essentially in cold conditions; e.g., energy savings and storage for managing reproductive capacities, development during the life cycle, and fecundity (Hovanitz, 1950; Graham et al., 1980; Boggs, 1981; Nielsen and Watt, 1998; Limeri, 2017; Woronik and Wheat, 2017; Woronik et al., 2018). The benefits of the 'non-alba' orange morph are sexual attractiveness and mating preferences, including better discrimination performance against other white Pieridae (Graham et al., 1980; Watt, 1995; Nielsen and Watt, 1998; Kemp et al., 2006, Limeri, 2017). Environmental studies have stressed the importance of ecosystems and biotopes for colour development of *Colias croceus*, emphasizing the roles of nitrogen dietary intake by the larvae (Nielsen and Watt, 1998), of the altitude, latitude, and longitude (Hovanitz, 1950), and the temperature and photoperiods (Hoffmann, 1973, 1974).

We encountered an especially interesting and more complex case of polymorphism on the Azores islands in the colianid species *Colias croceus*. *C. croceus* is a migrant species, which has its expansion centre around the Mediterranean basin (Leestmans, 1974). It is present in North Africa, in the Middle-East up to Iran, and all across Europe except northern Scandinavia. In the Macaronesian Islands, it is observed not only at the Azores, but also in Madeira, the Canary Islands, and Cape Verde (Tennent and Russell, 2015). No *bona* subspecies have been reported, although the Synonymic List of the Genus *Colias*, considering the rules of the International Commission on Zoological Nomenclature, provided 183 descriptions of invalid *Colias croceus* taxa, mostly forms and aberrations (Grieshuber and Lamas, 2007). In the Azores, the six different observed *croceus* taxa are the forms: ♂ and ♀ *croceus*, ♀ *helice* (Hübner, 1805), ♂ and ♀ *cremonae* (Verity, 1911), and ♀ *cremonaehelice* (Russell et al., 2003).

The colouration of pierid butterflies is prominently determined by pigments of the pterins group, which are specifically expressed in granules adorning the cross-ribs of the wing scales (Yagi, 1954; Nijhout, 1991; Ghiradella, 1998, 2010; Giraldo and Stavenga, 2007; Morehouse et al., 2007). The most abundant pterin pigments are leucopterin, xanthopterin and erythropterin, absorbing in the ultraviolet, violet and blue wavelength ranges, respectively, thus causing white, yellow, or orange/red wing areas (Descimon, 1975; Stavenga et al., 2006; Wijnen et al., 2007). For instance, the white wings of the '*alba*' morphs of pierids have very low xanthopterin and erythropterin levels (Descimon,

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1966; Watt, 1973; Graham et al., 1980).

The different colour phenotypes of the various *C. croceus* morphs indicate that the expression of the pterin pigments is variable, but structural factors might also be involved (Rutowski et al., 2007; Kemp and Rutowski, 2011). To characterize the pigmentary and possibly structural basis of the different wing colours, we applied a number of optical methods. We here report that the colours of individual scales depend on the differential expression of pterin pigments as well as on the thin film reflection properties of the scales' lower lamina. Wing colouration is the culmination of scale structure, pigmentation and organization, including scale stacking.

## 2. Materials and methods

### 2.1. Butterflies

We made observations and collected specimens during July and August 2010 and 2017 on three islands of the Azores, São Miguel, Faial and Flores. *Colias croceus* was present on all three islands, but the forms *cremonae* and *cremonaehelice* were only observed on Faial. RGB as well as UV photographs of pinned specimens were made with a Nikon D70 digital camera, which has a red channel with substantial UV sensitivity. For UV photography, two 15 W blacklights were used, and the camera lens (AF Micro Nikkor, 60 mm, 1:2.8 D) was fitted with UV-transmission and red-blocking filters (3 mm UG1 and BG38; Schott, Darmstadt, Germany). The light source for RGB photography was the Nikon flash unit Speedlight SB-800.

### 2.2. Spectrophotometry

Reflectance spectra of different areas of the wings were measured with a bifurcated probe (Avantes FCR 7–UV–200), using an AvaSpec 2048–CCD detector array spectrometer (Avantes, Apeldoorn, the Netherlands). The light source was a deuterium-halogen lamp (AvaLight D(H) S), and the reference was a white diffuse reflectance tile (Avantes WS–2).

Reflectance spectra of isolated scales were measured with a microspectrophotometer (MSP). The MSP was a Leitz Ortholux microscope (Leitz, Wetzlar, Germany) with an Olympus 20x, NA 0.46 objective (Olympus, Tokyo, Japan) and a xenon arc lamp as light source. The MSP collected the reflected light from effectively a square area with edge length 5–10 μm, by imaging a square diaphragm in the microscope's image plane at the entrance of an optical fibre connected to the detector array spectrometer; the white diffuse reflectance tile was also here used as a reference. Due to the glass optics in the microscope, the MSP spectra were limited to wavelengths greater than 350 nm. The applied epi-illumination had an about normal direction, and, as the scale's thin film reflections are directional and the reference is a diffuser, this caused overestimated reflectance values, which were corrected by an estimated factor 3. Absorbance spectra of isolated scales immersed in immersion oil (refractive index $n = 1.515$), were also measured with the MSP.

### 2.3. Imaging scatterometry of single wing scales

For investigating the spatial reflection characteristics of the scales, we performed imaging scatterometry (Stavenga et al., 2009; Vukusic and Stavenga, 2009; Wilts et al., 2009). A scale attached to a glass micropipette was positioned at the first focal point of the ellipsoidal mirror of the imaging scatterometer. The scatterograms were obtained by focusing a white light beam with a narrow aperture ($< 5^{\circ}$) at a small circular area (diameter 13 μm), and the spatial distribution of the far-field scattered light was then monitored. The exposure times of the scatterograms were appropriately adjusted so as to obtain an image with maximal contrast.

### 2.4. Modelling the reflectance spectra of thin films

The reflectance spectra of chitinous optical thin films in air were calculated for normally incident light using an expression derived from the classical Airy treatment (Yeh, 2005; Stavenga, 2014). A thin film can be considered as a medium with index number 1, between two media with index numbers 0 and 2. The reflection coefficients of the two interfaces $j = 1$ and 2, $r_{01}$ and $r_{12}$, for normal illumination are given by the Fresnel formula

$$r_{j-1,j} = (n_{j-1} - n_j)/(n_{j-1} + n_j) \quad (1)$$

where $n_0$, $n_1$, and $n_2$ are the refractive indices of the three media. The Airy formula for the reflection coefficient of the thin film is

$$r = [r_{01} + r_{12}\exp(-2i\varphi)]/[1 + r_{01}r_{12}\exp(-2i\varphi)]] \quad (2)$$

where for normal illumination $\varphi = 2\pi n_1 d/\lambda$, with $d$ the thickness of the thin film and λ the light wavelength. The reflectance of the thin film is obtained from $R = |r|^2$. For the lower lamina of the scales, media 0 and 2 are air; medium 1 is chitin, for which the refractive index is given by the Cauchy equation $n(\lambda) = A + B\lambda^{-2}$, with $A = 1.517$ and $B = 8.80{\cdot}10^3$ nm$^2$ (Leertouwer et al., 2011).

## 3. Results

### 3.1. Appearance of different phenotypic forms

In the Azores, 6 distinct phenotypic forms of *Colias croceus* can be observed: 1. Yellow-orange form, with orange discal spots on the dorsal surface of the hindwing, red legs, antennae, fringes and thoracic hairs, *croceus* ♂ (Fig. 1A); 2. Idem, *croceus* ♀ (Fig. 1B); 3. Lemon-yellow form, without any trace of red colouration in the discal spots on the dorsal surface of the hindwing, legs, antennae, fringes and thoracic hairs, *cremonae* ♂ (Fig. 1C); 4. Idem, *cremonae* ♀ (Fig. 1D); 5. Creamy white form, with orange discal spots on the dorsal surface of the hindwing, red legs, antennae, fringes and thoracic hairs, *helice* ♀ (Fig. 1E); 6. White form, with greenish-white discal spots at the upperside hindwing, legs, antennae, fringes and thoracic hairs, *cremonaehelice* ♀ (Fig. 1F). UV photographs demonstrate that the dorsal surface of the hindwings of the males is distinctly UV reflecting (Fig. 1).

### 3.2. Wing reflectance spectra

Previous studies inferred that the different appearance of the various morphs is due to differences in the pterin pigmentation of the wing scales and their spatial organization (Descimon, 1975; Stavenga et al., 2006; Wijnen et al., 2007). To characterize the wing colouration more quantitatively, we performed reflectance spectrophotometry with a bifurcated optical probe on 5 distinct wing locations (Fig. 2). Generally, the wing reflectance is low in the short-wavelength range and high in the longer wavelengths. The wavelength where the reflectance change is halfway ($\lambda_h$), is in locations #1 and #2 of both *C. croceus* ♂ and ♀ ~540 nm (Fig. 2C, D) and of both *C. cremonae* ♂ and ♀ ~495 nm (Fig. 2E, F); for *C. helice* ♀, $\lambda_h$ in locations #1 and 2 is ~430 nm and ~540 nm, respectively (Fig. 2G), and for C. *cremonaehelice* ♀ $\lambda_h$ is ~430 nm (Fig. 2H). Similar transition wavelengths were encountered in a previous study on several pierid butterflies where wing reflectance measurements were combined with absorbance measurements on pigments extracted from the wing scales (Wijnen et al., 2007).

Corresponding to the UV images, which show distinct UV-reflections (see also Pirih et al., 2011), the reflectance spectra of location #3 of the two male forms show a clear reflectance band in the short wavelength range (Fig. 1A,C and 2C,E). This must be due to ridge lamellae that together create a multilayer reflecting in the ultraviolet wavelength range (Kemp et al., 2006). In the wing margin (location #4), the reflectance is low, slightly increasing with increasing wavelength. The latter is characteristic for media containing melanin pigment. The

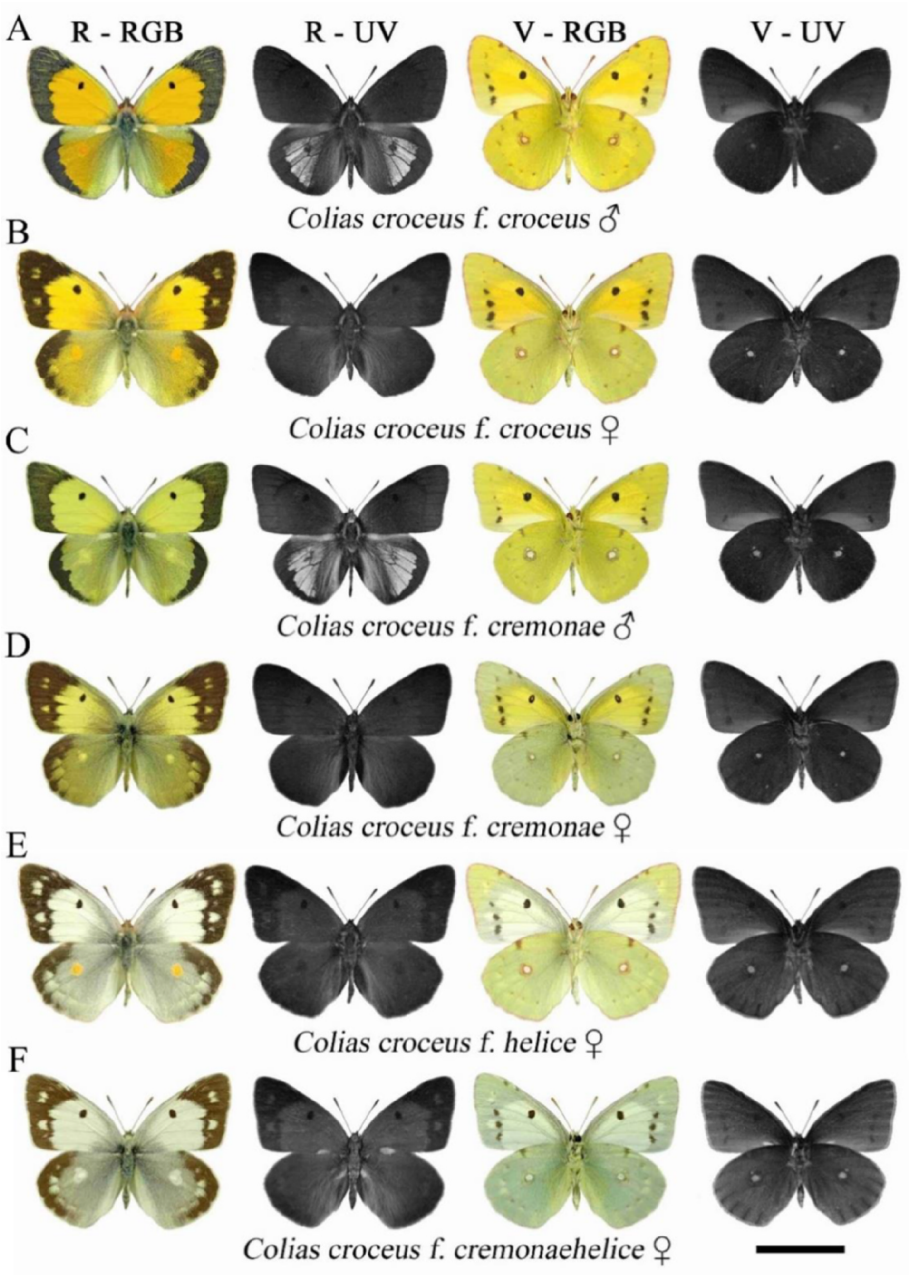


**Fig. 1.** Polymorphism of *Colias croceus* observed in the Azores. (A) *C. c. f. croceus* male. (B) *C. c. f. croceus* female. (C) *C. c. f. cremonae* male. (D) *C. c. f. cremonae* female. (E) *C. c. f. helice* female. (F) *C. c. f. cremonaehelice* female. R – recto, V – verso, RGB – colour image, UV – ultraviolet image; scale bar: 2 cm.

reflectance of location #5, the discal spot on the ventral surface of the hindwing, is rather high at all wavelengths. This high reflectance means a low absorbance, or a minor amount of pigment. Apparently, the pigmentation here is distinctly less than in the other wing regions (see further below).

### 3.3. Difference in colouration of the two sides of the wing scales

Since the wing colour pattern is created by the lattice of scales adorning the wings, we inspected single wing scales from different wing areas. This revealed the decidedly different appearance of individual, isolated scales as well as the different appearance of both scale sides (Fig. 3). Firstly, the upper or abwing side of the scales is matte and the under or adwing side is mostly shiny (Fig. 3, left column). This difference in appearance, well known for most butterfly wing scales, is intimately related to the scale anatomy (Ghiradella, 1998; Stavenga et al., 2014). As the upper lamina consists of ridges connected by cross-ribs with scattering granulae, epi-illumination of the upper (abwing) side of a scale creates diffusely scattered light, which is selectively filtered by the pigment. The colour of the scattered light is thus determined by the pigment's absorption spectrum. For instance, in orange scales (from area #1), the short-wavelengths-absorbing pterin pigment (Fig. 4A, B) suppresses the reflection in the short wavelength range so that the remaining, spatially-widespread reflection (Fig. 3A, upper scatterogram) results in an orange colour, or, the reflectance spectrum is high in the long wavelength range (Fig. 3A, right column). Quite differently, as the lower lamina is a thin film, epi-illumination of the scale's adwing side causes a glossy image (Fig. 3A, lower left image) and a spatially very restricted reflection pattern, characteristic for a specular surface (Fig. 3A, lower image of middle column). The reflectance spectrum of the adwing side (Fig. 3A, right) resembles that of a chitinous thin film, and modelling indicates a thickness of 130 nm (Fig. 4C).

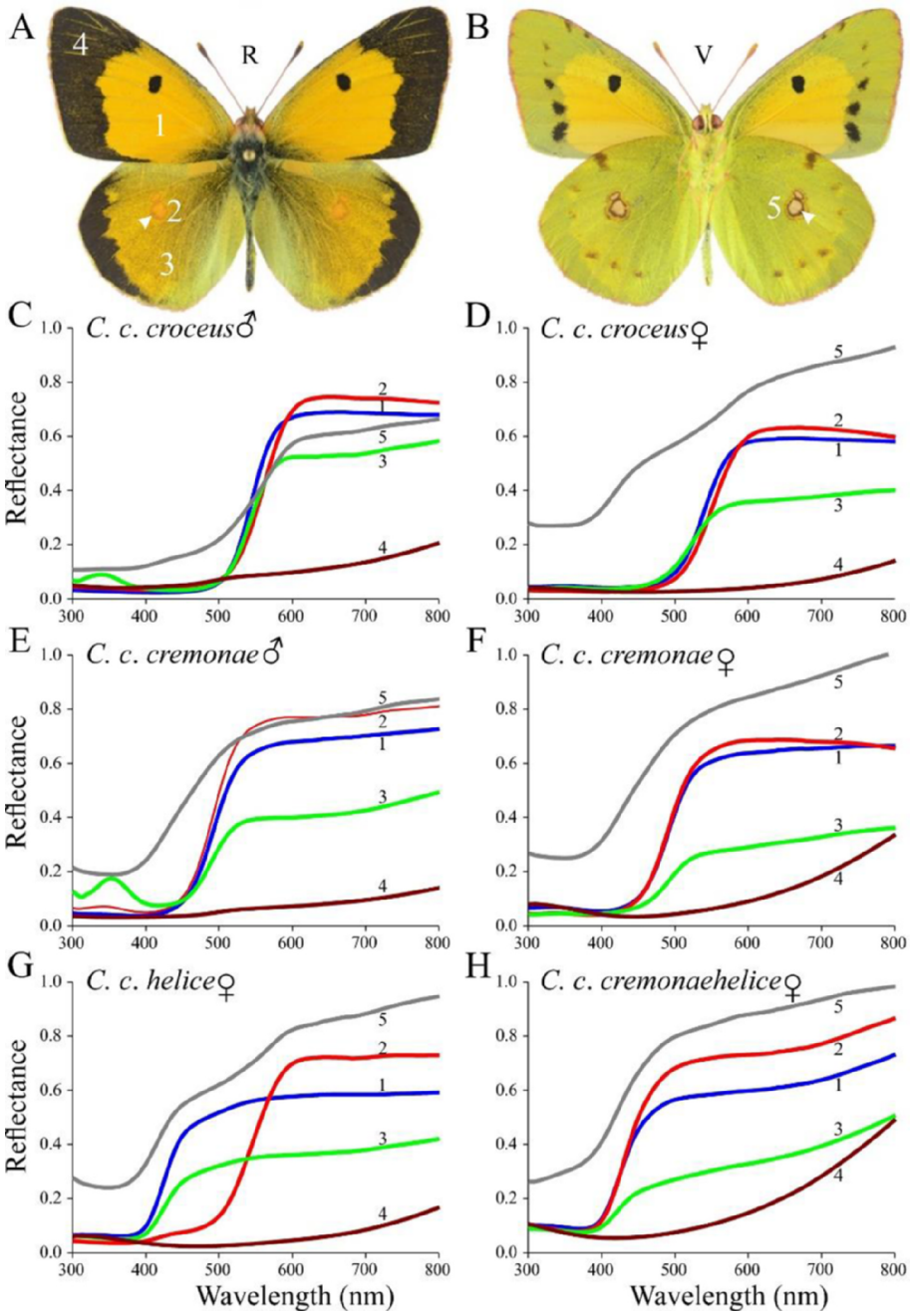


**Fig. 2.** Reflectance spectra of five characteristic locations of the wings of *C. croceus* forms. (A, B) Measurement locations 1–4 on the upperside and location 5 on the underside of the wings, shown in the case of a male *C. c. croceus*. (C–H) Reflectance spectra of the five wing locations of the different forms measured with a bifurcated reflection probe with a white diffuse reflectance tile as reference.

The wing margins, area #4, are brown-black and have a low reflectance (Fig. 2, #4). Absorbance measurements of the marginal cover and ground scales demonstrate that they contain ample melanin pigment (Fig. 4A). Curiously, the reflectance spectra of the adwing sides strongly differ. Modelling predicts that the thickness of the lower lamina of the cover scales is ~130 nm, whereas the thickness of the ground scales is predicted to be ~210 nm (Fig. 3B,C, right column #4; Fig. 4C).

Epi-illumination of the abwing side of a whitish scale from area #5 produces a scatterogram with a multi-coloured line. The latter is due to diffraction by the array of parallel ridges. The minor background is due to backscattering by the cross-ribs connecting the ridges of the upper lamina. The adwing side of the scale is somewhat wrinkled (Fig. 3D, lower left), resulting in a scatterogram showing a somewhat spread-out spatial distribution. In agreement with the rather colourless appearance of the scale, transmission microspectrophotometry showed the absence of absorbing pigments.

### 3.4. Scale stacking

The colouration and reflectance spectra of the wing is the collective result of the reflection properties of the tapestry of scales that covers the wing substrate (Fig. 5). For instance, in Fig. 5A, taken from area #3 in the dorsal (upperside) hindwing, orange cover scales are backed by brown ground scales. The reflectance thus is due to a mixture of scales with pterin and melanin pigmentation (Fig. 5A). Where orange scales overlap each other, the colour is more saturated, showing that part of the incident light travels through the cover scales and then, when

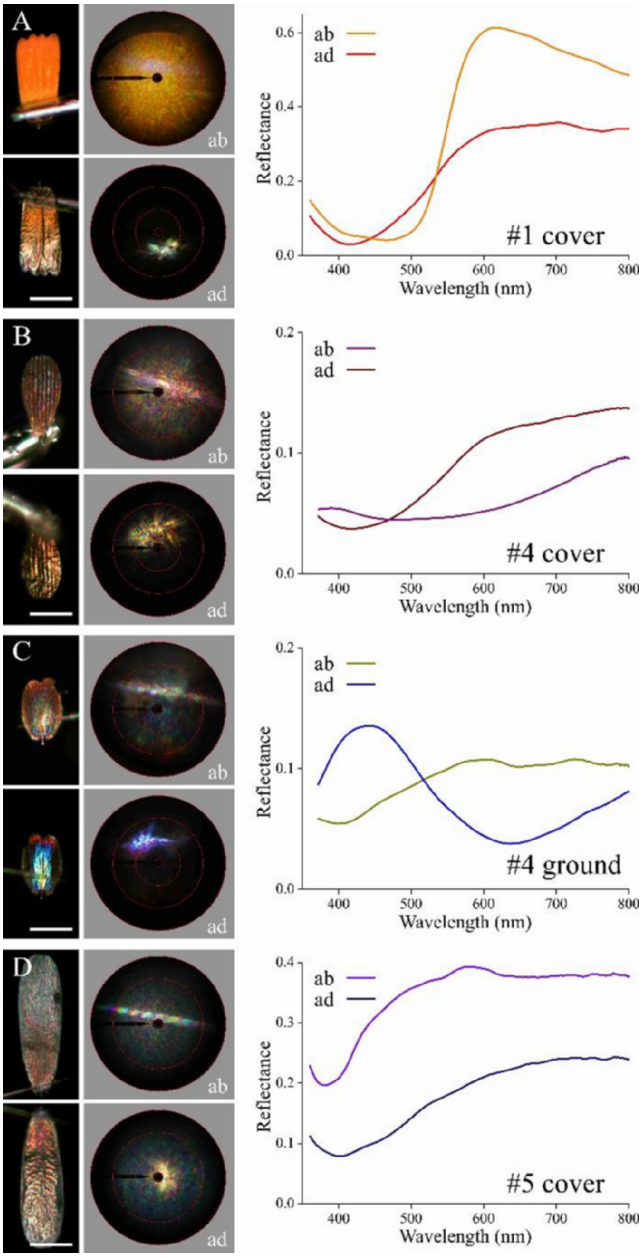


**Fig. 3.** Optical characteristics of scales from different wing areas of a male *C. c. croceus* attached to a glass micropipette rod. (A) A cover scale from the orange, dorsal forewing (#1). (B) A cover scale from the brown-black dorsal margin (#4). (C) A ground scale from the dorsal margin (#4). (D) A whitish cover scale from the eye spot of the ventral hind wing (#5). Left column: upper photograph – abwing side; lower photograph – adwing side; scale bar: 50 μm. Middle column: upper scatterogram – abwing side (ab); lower scatterogram – adwing side (ad). Right column: reflectance spectra measured with a micro-spectrophotometer. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

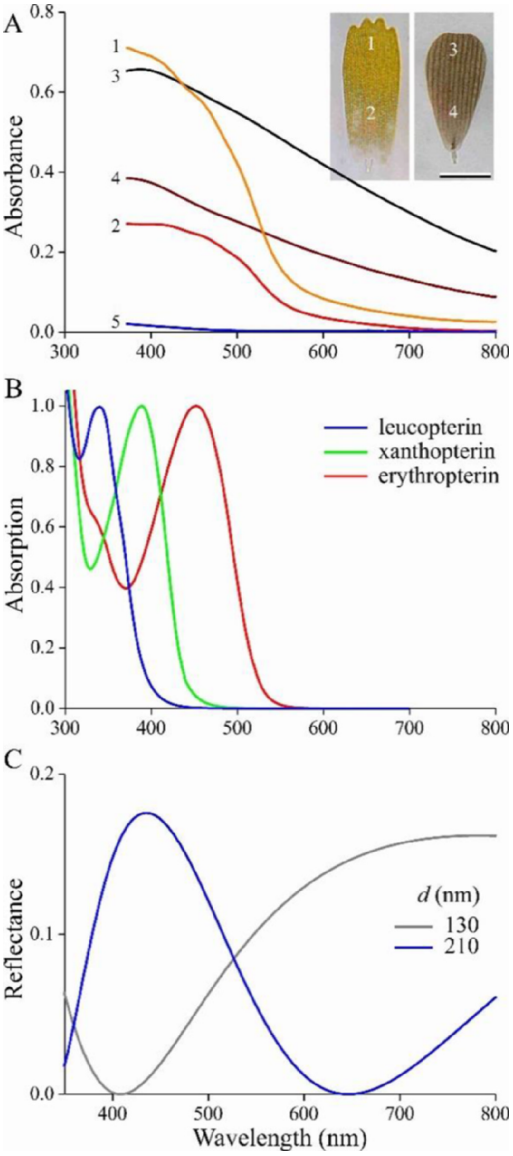


**Fig. 4.** Absorbance spectra of scales and wing and thin film reflectance spectra. (A) Absorbance spectra of tip and root areas of an isolated orange (1, 2; see inset) and brown-black (3, 4; see inset) scale in immersion oil, as well as of a denuded wing (5); scale bar inset: 50 μm. (B) Normalised absorption spectra of pterins (from Wijnen et al., 2007). (C) Reflectance spectra of thin films with thickness (*d*) 130 nm and 210 nm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

reflected by the ground scale and subsequently having travelled back through the cover scale, adds to the total reflectance. The wing colouration is hence due to the reflectance of the stack of cover and ground scales. In locations #1 and 2, both cover and ground scales are pterin pigmented, while in area #3 the ground scales contain melanin, yielding a less pronounced overall reflectance (Fig. 2C–H).

The individual scales in the rim of the eyespot in the ventral (underside) hindwing have a rather uniform colour (Fig. 5B). This demonstrates that the pigment expression is characteristic for the scale, but the colour of neighbouring scales can clearly be quite different. The colour of a wing area with differently coloured scales is therefore a mixture of the various scale colours.

Fig. 5C presents a damaged wing area of the dorsal forewing where locally both cover and ground scales were removed. Below the transparent wing substrate, the ground scales at the wing underside can then be seen, but normally, when the wing's scale stack is intact, the blue reflection of the adwing side of the latter scales is fully blocked (for reflectance spectra due to contributions by scales from both wing sides, see Stavenga et al., 2006).

## 4. Discussion

*Colias croceus* is a migratory, common species, with high dispersing capacity, having established permanent populations on various islands of the Azores (Leestmans, 1974; Tennent and Russell, 2015). This butterfly species classically has a particularly low genetic

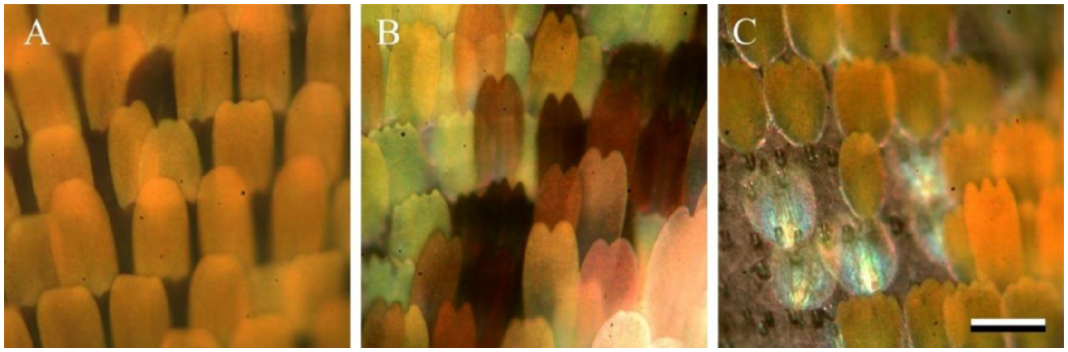


**Fig. 5.** Scale lattice in various wing areas of *C. c. croceus* male. (A) Dorsal hindwing area (#3, Fig. 2A). (B) Rim area of eyespot in dorsal hindwing (#2, Fig. 2A). (C) Damaged dorsal forewing area (#1, Fig. 2A). Scale bar: 50 μm.

differentiation between populations, but the situation is very special in the Azores, where six different forms of *croceus* are observed (Russell et al., 2003).

Three of these, *f. croceus* ♂ and ♀, and *f. helice* ♀, are consistent with the patterns of the well-known '*alba*' polymorphism, observed in other *Colias* species more than two hundred years ago. The '*alba*' form of *croceus* was described as *f. helice* (Hübner, 1805). In *croceus*, the males display the same colour as the non–'*alba*' females (orange), while the '*alba*' females are paler (whitish) (Fig. 1). In Coliadinae, the '*alba*' polymorphism is ubiquitous and ancestral, but instable and can be transitory, perhaps due to environmental factors (climate, host plants and symbiotic species) (Limeri and Morehouse, 2016; Limeri, 2017).

Genetically, the '*alba*' morph phenotype can be expressed when the '*alba*' gene is transmitted as a sex limited (only ♀ exhibit the phenotype), dominant autosomal trait (only one parent has to transmit the gene) (Remington, 1954; Lorković and Herman, 1961). In *croceus*, genetic studies have mapped the '*alba*' gene to a single locus and candidate gene, *BarH1*. Differences at the *BarH-1* locus likely impact the regulation of downstream genes responsible for the expression of the '*alba*' phenotype (Woronik, 2017; Woronik and Wheat, 2017; Woronik et al., 2018).

Biochemically, the '*alba*' phenotype translates into modifications in the biosynthesis pathway of the pterins (Fig. 6). The '*alba*' morph is characterised by very low pterin levels, essentially concerning xanthopterin and erythropterin (Descimon, 1966; Watt, 1973). Electron microscopy confirmed the reduced level of pterin pigment grains in the '*alba*' female wings (Woronik, 2017). The spectral characteristics of the various morphs indicates the predominant role of erythropterin in non–'*alba*' *f. croceus* ♂ and ♀, and of leucopterin in '*alba*' *f. helice*. The level of the limiting action of the '*alba*' gene in the biosynthesis pathway remains uncertain.

In the '*alba*' polymorphism, regulatory genes could be modulated by environmental factors (Fig. 7). In some *Colias* species (*meadi*, *alexandra*, *scudderi*), which express the '*alba*' phenotype, the percentage of '*alba*' females in the populations seems to depend on abiotic factors as temperature, humidity, altitude, or nitrogen content in the hostplants (Watt, 1973; Nielsen et al., 1988). The same abiotic factors can also influence the level of expression of pterins, thus modulating the colours within the phenotypic range typical of the species form. Environmental studies have stressed the importance of ecosystems and biotopes for colour development of *Colias croceus*. Dietary intake by the larvae, particularly of nitrogen, depends on many factors (Nielsen et al., 1998). For instance, altitude, latitude, longitude (Hovanitz, 1950), temperature and photoperiods (Hoffmann, 1973, 1974) regulate the biosynthesis of the different pterins involved in imago chemical colouration and the elaboration of very sophisticated chitinous wing structures, which also underlie the physical colouration in imagos.

The '*alba*' polymorphism is maintained by different selective pressure balances. Pterins are extremely nitrogen-rich pigments. For growth and sexual selection, butterflies are nitrogen-limited, depending on nitrogen-containing nectar (food sources with high concentration of amino-acids). Honest signalling theory posits that only high-quality individuals can sequester enough nitrogen for allocation in the brain, wing muscles, reproductive tissues and the wings (Zahavi, 1975; Morehouse and Rutowski, 2010; Espeset, 2019). More brightly coloured (males and females) and particularly UV-reflecting specimens (males) are sexually more attractive, because of an honest signal of reproductive quality, but have to spend more energy to achieve this (Kemp and Rutowski, 2011). The living conditions of other specimens oblige them to favour saving and storing energy for managing their reproductive capacities, eggs, larvae and pupae development, longevity and fecundity of imagos (reallocation of resources) (Graham et al., 1980; Boggs, 1981; Nielsen et al., 1998; Limeri, 2017; Woronik, 2017). Maintenance of reserves seems to be to the detriment of sexual attractiveness (Graham et al., 1980; Watt, 1995; Kemp et al., 2006). In particular orange non–'*alba*' males or females (*croceus*) living as larvae in short daylength growth conditions have reduced erythropterin levels and are thus more yellowish, while white '*alba*' females (*helice* and *cremonaehelice*) can become yellowish or pinkish after cold growth conditions. We found that this phenomenon is less apparent in specimens bearing the '*cremonae*' phenotype, presumably due to the complete lack of erythropterin in these specimens. Furthermore, '*non-alba*' males growing in short daylength are also less UV-reflecting, indicating scale structural differences (probably fewer overlapping lamellae in the ridges). Analysing RGB and UV pictures of several specimens captured during the same period but on different biotopes on Faial island, we observed that the brightest coloured orange males were also the most UV-reflecting, indicating perhaps a double investment of energy for gaining sexual attractiveness. The parallelism of brightness-UV reflectance was not present in *croceus* females, as they are never UV-iridescent. In the genetically-close African *Colias electo*, the brightest orange females show also the strongest UV-iridescence. Short daylength and cold growth conditions, along with less energy stores, could generate less brightly coloured and mainly less UV-iridescent specimens, which thus are less sexually attractive (Fig. 7). As the weather is very changeable in the Azores islands, and temperatures can vary considerably on the same day, specifically designed field studies will be necessary to understand the interference between "local" climate and specimens' phenotypes.

We observed in the Azores three "classical" forms, non–'*alba*' *croceus* ♂ and ♀ and '*alba*' *helice*, with orange (*croceus*) and whitish (*helice*) ground colours. The dorsal hindwing discal spots, legs, antennae, thoracic hairs and fringes contain orange/red pigment. For explaining the three additional observed forms, a second mechanism must come into play and add its effects to those of the '*alba*' gene. We observed no orange/red pigment, neither on the background nor on the dorsal hindwing discal spots, legs, antennae, thoracic hairs and fringes. Apart from this absence of red pigment, *f. cremonae* ♂ and ♀ and

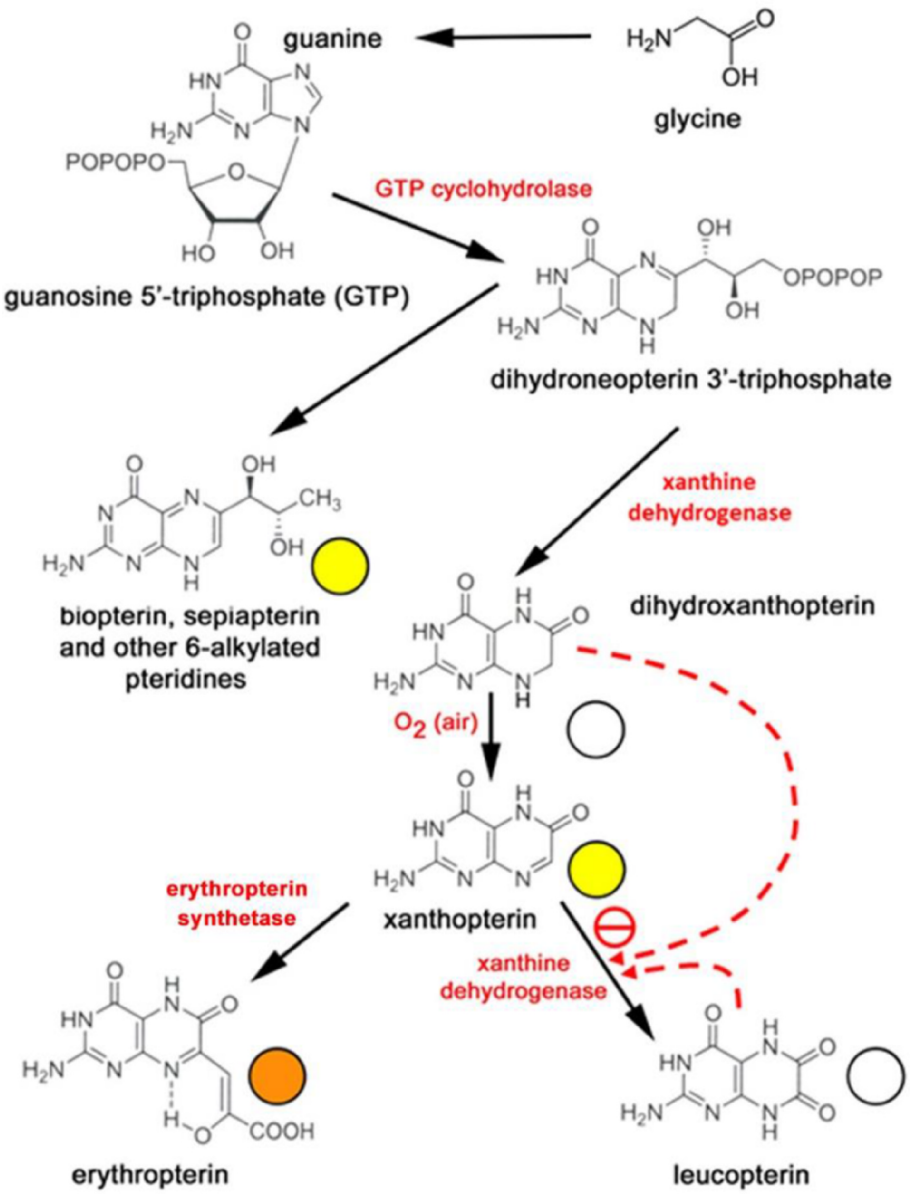


**Fig. 6.** Diagram of pterin biosynthesis pathway. Dotted red lines represent competitive inhibition of the xanthine dehydrogenase enzyme (Watt, 1967; 1972; Descimon, 1973; Morgan, 2010; Shamim et al., 2014). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

*cremonaehelice* ♀ appear to correlate with "classical" non–'*alba*' *croceus* ♂ and ♀ and '*alba*' *helice*, respectively. The mechanism could be environmental or genetic (Fig. 8).

With the exception of populations isolated in the Azores, yellow-coloured *Colias croceus* are very rare elsewhere in their distribution area. A taxon has been described from Romania as *Colias croceus f. erateformis* Niculescu, 1963, and isolated specimens were encountered in Cyprus (John et al. 2006), Greece, Rhodes (Cuvelier and Schneider, 2012) and Turkey (Fig. S1). Their ground colour is yellow, in contrast with all the specimens of *f. croceus* and *helice* observed and illustrated in Fig. 1A,B,E, but orange-red pigments are present in the upperside hindwing ocellae as well as in fringes, thoracic hairs, antennae and legs (Fig. S1B,C). Thus, abiotic factors influence the balance between nitrogen savings and sexual attractiveness, modifying not only quantitatively and qualitatively the pterin levels but also the development of chitinous photonic structures in the wings (Kemp, 2008; Fenner et al., 2019). In contrast, these factors seem not sufficient to explain the total absence of red pigment observed in *f. cremonae* and *cremonaehelice*.

The *cremonae* and *cremonaehelice* forms are present essentially on only one island (Faial), although it is very close to other islands of the Central Group of the Azores archipelago. In some observation data, these forms have reached more than 6% of populations, ranging to sometimes thousands of individuals (Russell et al., 2003). One explanation could be that genetic isolation of these populations, their consanguinity, can result in a gradual increase of the expression of recessive alleles. We therefore hypothesize that the *cremonae* and *cremonaehelice* forms of *Colias croceus* are related to the expression of another genetic polymorphism, which we call *cremonae*, a name initially chosen for the form. The common characteristic of these two forms lies in the absence of an orange-red colour in the wing scales, fringes, hairs, antennae and ocelli of the imagos. The gene involved seems to be recessive and non-sex-linked (Russell et al., 2003). Gene expression is also probably modulated by abiotic factors, explaining the large panel of morphs observed in Faial.

Our spectrophotometry indicates the absence of erythropterin in the wing scales of the *cremonae* ♂ and ♀ and *cremonaehelice* ♀ analysed

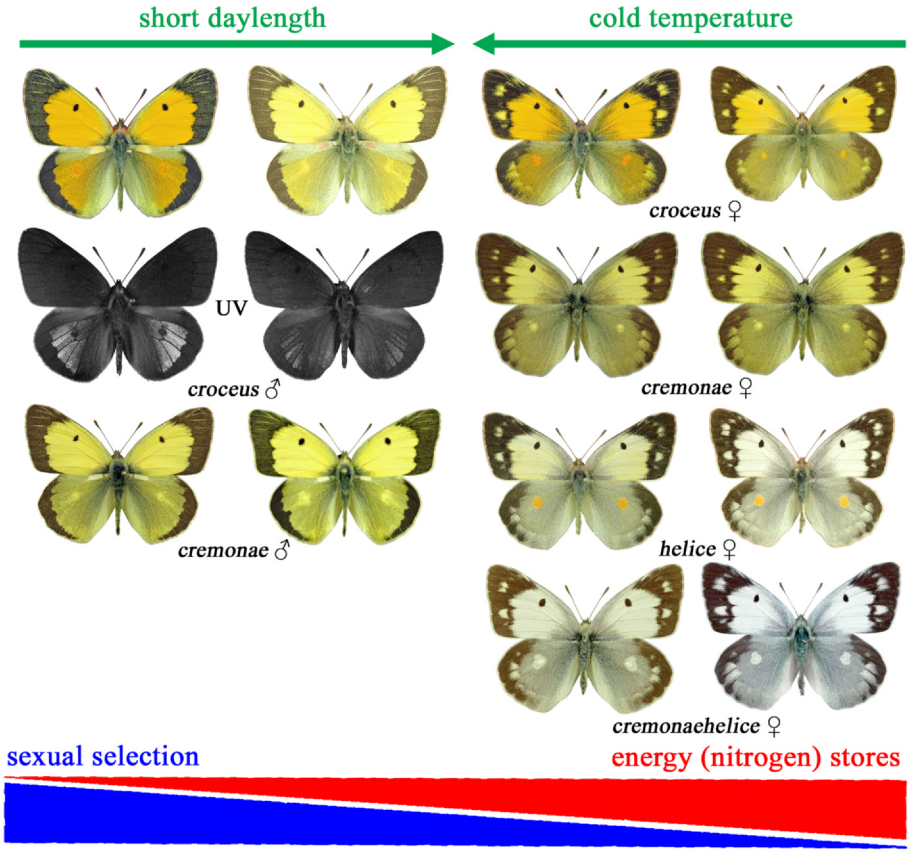


**Fig. 7.** The role of environmental factors in various morphs of the different forms in *Colias croceus* and potential balance between advantages of sexual attractiveness (male and female bright colour pattern and high UV-reflectance based choice in sexual selection) and nitrogen stores (limited by energetic costs of honest signalling).

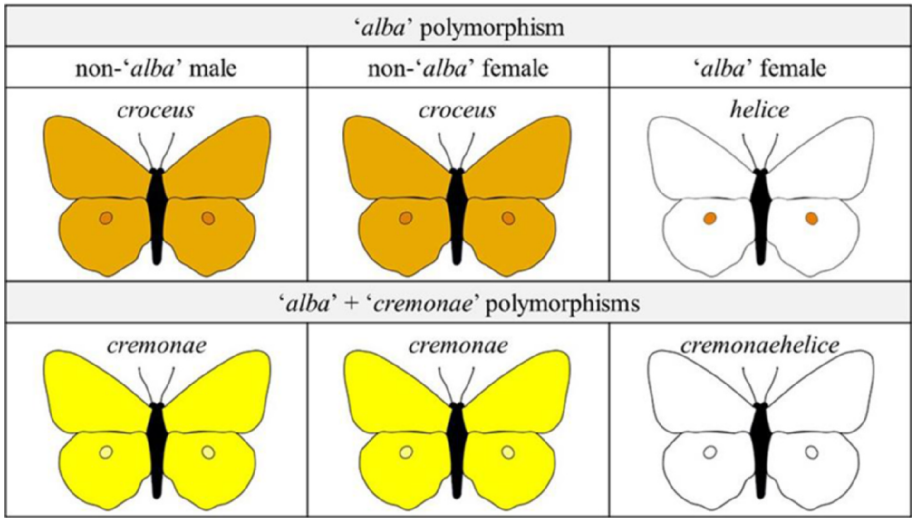


**Fig. 8.** Combined action of '*alba*' and '*cremonae*' polymorphism in the different *Colias croceus* forms observed in the Azores. The '*cremonae*' polymorphism fully suppresses the expression of orange/red pigment in the main wing areas as well as in the dorsal hindwing discal spots, legs, antennae, thoracic hairs and fringes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

specimens. We hypothesize that the expression of a gene involved in the biosynthetic pathway of pterins is blocked at the level of the enzymatic transformation of xanthopterin into erythropterin (erythropterin synthetase; Fig. 6). Further biochemical and genetic studies are necessary to clarify this point.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jinsphys.2020.104114.

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