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Colored feathers in museum collections: a spectroscopic study of 3 bio-pigments and their lightfastness

Céline Daher¹, Aurélie Tournié¹, Fabrice Sauvagnargues², Christine Andraud¹, Jacques Cuisin³,
Véronique Illes⁴, Éléonore Kissel²

¹ Centre de Recherche sur la Conservation (CRC, USR 3224), Muséum national d'histoire naturelle,
Ministère de la Culture, CNRS ; CP21, 36 rue Geoffroy-Saint-Hilaire, 75005 Paris, France

² Musée du quai Branly - Jacques Chirac, 222 rue de l'Université, 75007 Paris, France

³ Muséum national d'histoire naturelle, CP23, 57 rue Cuvier, 75231 Paris cedex 05, France

⁴ Centre de Recherche et de Restauration des Musées de France (C2RMF), Petite écurie du roi, 2
avenue Rockefeller – CS 50505, 78000 Versailles, France

Abstract

Yellow to red feathers in ornithological and ethnographical museum collections are known to be sensitive to light exposure. However, only a few studies on the interaction of light with the color of feathers have been published. This work provides fresh insight into the sensitivity of feathers for better conservation and exhibit of ethnographic collections, with a specific focus on carotenoids, psittacofulvins and porphyrins-based feathers. A first approach was to evaluate the use of Fiber Optics Reflectance Spectroscopy (FORS) in the visible range to characterize the chemical nature of the bio-pigments. It was implemented on 19 ornithological specimens preserved in the *Muséum national d'histoire naturelle (MNHN)* in Paris, and on assigned feathers from more than 40 objects from the collections of the *musée du quai Branly - Jacques Chirac (mqB-JC)*. Then, microfading tests were carried out on molting feathers or picked up on dead birds. Two different setups were used in order to determine a reproducible protocol for these 3D materials. Several variables were considered including the positioning of the feather and the duration of measurements. In addition to the visible range, the near-infrared (NIR) region was also investigated to determine if the color fading is concomitant to any structural modification. Then the developed protocol was applied on some loose lumps of feathers from objects of the *mqB-JC* collections. The data were compared against each other and to the Blue Wool Standards fading rates.

Keywords

Microfading test; color change; colorimetry; FORS; feathers; bio-pigments

1. Research aims

This research aims to compare the lightfastness of three bio-pigments (carotenoids, psittacofulvins and porphyrins) that are found in yellow to red feathers in ethnographic collections using microfading tests (MFT). An analytical procedure was developed on a set of loose feathers in order to determine the optimal conditions of analysis. The feasibility of this approach was then assessed on feathers from museum objects. Further insights into the color changes during the fading process are provided.

2. Introduction

Lighting in museums is a very challenging task as it must cover three important concepts: the scenography of the exhibition, the visual comfort of the visitors and the conservation of the exhibited objects. This last notion is a major concern for cultural heritage scientists, whose tasks are to evaluate the alteration factors such as photodegradation, and to inform on the optimal conditions of lighting. For that purpose, Paul Whitmore introduced in 1999 [1] an innovative technique he referred to as the microfading tester, to evaluate the fading of an artwork during an exhibition. Since then, microfading testing (MFT) has been widely used on flat and opaque artifacts: artworks on paper such as pastels [2], drawings or documents containing iron gall inks [3], art materials [1,4], and photographs [5]; as well as more textured colored objects: textiles [6–8], Alaskan artefacts [9] and tapestry [10]. Ford and Druzik [11] have specified the use, advantages and limitations of MFT with a very detailed review. This technique consists in intensively illuminating a tiny surface of an artwork (less than 0.5 mm in diameter) with a light source operating in the visible range, and simultaneously following the spectral change, which is later converted to color change (ΔE) in a specific colorimetric space (here CIELAB, defined in 1976 by the *Commission Internationale de l'Éclairage (CIE)* [12]). The potential color modification is compared to the fading of normalized references, Blue Wool Standards (BWS) of varying lightfastness (from BWS1 the most sensitive, to BWS8 the less sensitive; typically, microfading tests use BWS1-3), that are tested under the same illuminating conditions. The Just Noticeable Difference (JND) is a criterion introduced to determine the color change that is visible to an observer, with average color perception under ideal viewing daylight conditions. It is obtained by comparing side by side middle sized, smoothed, opaque and evenly colored materials. Reported average values for the characterization of a JND are $\Delta E_{94}=1.7$ [13], $\Delta E_{76}=1.8$ [14], $\Delta E_{00}=1.6$ [11]; the corresponding exposures inducing the JND are given in Mlx.h .

1 The objective of this study was to get more insight into the use of MFT for poorly characterized
2 objects such as feathers in ethnographic collections. Feathers are curved and textured items and can
3 sometimes be very thin and fragile. Their various colors can be of different origins: physical and/or
4 chemical [15]. Physical colors can be due to the interference of light: the barbules contain
5 microlamellar structures that break down the light, and depending on the spacing of the
6 microlamellae, the color differs. They can also be due to a spongy structure located in the barbs,
7 constituted of air cells regularly distributed in the keratin structure causing the reflection of short
8 wavelengths (blue and violet) [16]. Regarding the chemical colors, the most common pigment is
9 melanin responsible for brown and black colors which are stable to light. Carotenoids are responsible
10 for yellow, orange and red colors, and depend on the bird's diet [17]. Psittacofulvins are from
11 endogenous origin, and vary from yellow to red [18]. Porphyrins, also from endogenous origin,
12 produce several colors, as brown, red or green [18]. A given feather can present important variations
13 of color that may be due to the presence of a combination of physical and chemical colors, and/or a
14 combination of several bio-pigments in various concentrations and distribution. Among individuals of
15 a given species, the color variability of the feathers will depend on the age of the bird and the time of
16 year during which the feathers were collected. In museums, conservation conditions and past
17 exposure to light of feathered objects can also have an impact on their colors. The mechanical
18 damage due to handling, packing and transportation, and the biological damage (insects) will mainly
19 have an impact on physical colors through the degradation of the structure [19].

20 Although structural colors can degrade with UV radiation (degradation of keratin) [20,21] chemical
21 colors (i.e. bio-pigments) are sensitive to visible light. Few studies on the interaction of light with the
22 color of feathers have been published. Some studies are related to the identification and
23 differentiation of bird species using spectroscopic techniques such as visible reflectance spectroscopy
24 [19,22,23] or Raman spectroscopy [24–27]. Some other publications are related to the conservation
25 of museum artefacts by comparing MFT and window fading [20] or even the effects of exposure to
26 light and laser radiation during laser cleaning [28].

27 In ethnographic collections, the bird species from which the feathers have been taken to make the
28 objects – and hence the bio-pigments – are usually unidentified. In this work, only red to yellow
29 feathers containing carotenoids, psittacofulvins, or porphyrins were investigated, as they are
30 reputedly more sensitive to light exposure [28]. A first approach was to evaluate the use of Fiber
31 Optics Reflectance Spectroscopy (FORS) in the visible range to characterize the chemical nature of
32 the bio-pigments. It was implemented on a large corpus of 19 ornithological specimens of the
33 *Muséum national d'histoire naturelle (MNHN)* in Paris, and on assigned feathers from more than 40
34 objects from the collections of the *musée du quai Branly - Jacques Chirac (mqB-JC)*.

Microfading tests were carried out on molting feathers collected in menageries or picked up on dead birds. Two different setups were used in order to determine a reproducible protocol for these 3D materials. A comparison of two setups was conducted. This first device uses a xenon lamp and the second one, most commonly found in museums, uses LEDs. Multiple variables were studied, among them the positioning of the feather and the duration of measurements. The near-infrared (NIR) region was investigated in addition to the visible range in order to highlight possible structural modifications during the color fading. Then the developed protocol was applied on some loose lumps of feathers from objects of the *mqB-JC* collections. The data were compared against each other and to the BWS fading rates.

3. Material and methods

All samples and objects are described in Table 1, with the bird species they come from, their origins, their colors, and the type of bio-pigments they contain. The analyses that were performed for each sample or object are also indicated. Figure 1 presents images of the *mqB-JC* loose feathers, along with a specimen from the *MNHN*.

3.1. Feathers

3.1.1. Loose feathers and ornithological specimens

A total of 13 loose feathers consisting in molting feathers collected in menageries or picked up on dead birds were studied. They all date to the late 20th and 21st centuries. Most of them are yellow to orange with carotenoid pigments, three have psittacofulvin yellow pigments and one is red with porphyrin pigments. In addition, 19 specimens of the *MNHN* in Paris were analyzed. Mostly dated to the late 19th century, 13 specimens are with carotenoids-based colors (yellow and red), 5 with psittacofulvins-based colors (yellow and red) and one with red porphyrins.

3.1.2. Museum objects

A set of 44 feathered objects from the collections of the *mqB-JC* was analyzed. There are tunics and ponchos, headdresses, earrings, diadems, woven mats, bracelets, armlets, nasal ornaments, among others. They all come from South America and can be divided into two groups: archaeological (700-1450 AD) and ethnographical (1880's-1970's) collections. Their conservation state varies depending on their production period and former use, as well as on their conservation environment before and since entering the museum collections.

3.2. Spectroscopic study

3.2.1. Fiber optics reflectance spectroscopy (FORS)

FORS was performed using a FieldSpec Hi-4 (ASD Inc. Malvern Panalytical, Spectris plc, Egham, Surrey, UK), a spectrophotometer equipped with a halogen light source and a bundle of quartz-silica optical fibers transmitting in the visible and near-infrared wavelengths (350–2500 nm), connected to a contact probe-head operating in the 0°/45° geometry and a measuring spot size of approximately 6 mm². The spectrophotometer has 3 detectors to cover the whole spectral region: one for the 350–1000 nm range, another one for the 1000–1800 nm and the last one for the 1800–2500 nm. The spectral resolution is of 3 nm for the first range and 10 nm for the other two. The instrument is calibrated using a Spectralon® white reference, every 20 minutes approximately. Multiple spectra were recorded on several spots for each sample or object. Each spectrum is an average of 50 spectra with an acquisition time of 8.5 ms. FORS analyses were performed directly in contact with the samples or objects, and when the feather was very thin, a black cardboard was placed under it in order to absorb the light transmitted through the feather and prevent any back reflection.

3.2.2. Microfading Test (MFT)

The microfading test was performed using two different configurations: a LED setup developed by Pesme [29] and a slightly modified Whitmore setup [1]. For both instruments a set of Blue Wools Standards BWS1 to BWS5 (Deutsche Echtheits-kommission - DEK e.V.) was tested before each sample or object.

The LED setup is equipped with two LEDs: a warm white and a 405 nm light (Thorlab). The combination of both lights is brought to the sample with a 400 µm diameter optical fiber connected to an 800 µm diameter optical fiber through a shutter allowing to quickly stop the illumination when necessary. The estimated photometric intensity of the spot reaching the sample is 10 000 lx. The signal is collected in the 0°/45° geometry using a 400 µm optical fiber. In this setup, the measuring head, a semispherical angled fiber holder, is in contact with the sample and the analytical spot is approximately of 0.6 mm in diameter. The spectrometer is a Control Development LM-1000. The monitored reflectance spectrum is collected using the default settings: a 350–800 nm range, and an average of 10 spectra with an acquisition time of 5.8 ms. The color change induced by the illumination is calculated in real time (spectra are converted into CIELAB coordinates with D65 reference illuminant and 2° observer).

The Whitmore setup has optical and electronic components marketed by Newport: a xenon lamp (75 W) equipped with a UV filter, and a neutral density filter of 0.09 to deliver a photometric intensity at the sample between 0.3 and 0.5 Mlx. There was no IR filter in our experiment, as opposed to Whitmore's initial setup. A shutter is placed after the lamp and filters to quickly stop the illumination if needed. The light is brought to the sample with a 600 μm diameter optical fiber and the signal is collected in the $0^\circ/45^\circ$ geometry using the same bundle of fibers (without the contact probe) and the same spectrophotometer as for the FORS analyses. A series of lenses are used at the end of the fibers to focus the light on a small area (less than 0.5 mm in diameter) with a working distance of 12 mm. For a more precise focus, two crossed mini lasers were attached on each side of the measuring head: the focusing point is optimal when the two crosses meet (see Figure 3-b). The use of the FieldSpec Hi-4 spectrophotometer (and the absence of an IR filter) was intended to follow the infrared spectral range together with the visible range. The spectrometer tracks reflectance changes in real time, and the ΔE^*_{ab} [12] is monitored during the fading.

For both setups, MFT analyses were done on single feathers, and on loose feathers from the museum objects. A black cardboard was placed under the samples during the tests. The instrumental drift of the Whitmore setup was recorded on a Spectralon® white reference under the same conditions.

4. Results and discussion

4.1. Identification of bio-pigments by FORS in the visible range

In order to evaluate the use of FORS for the identification of bio-pigments, analyses were performed on 19 ornithological specimens of the *MNHN*, and on assigned feathers from more than 40 objects from the collections of the *mqB-JC*.

Figure 2 presents the reflectance spectra obtained on yellow (Y) feathers (Figure 2-a) and red (R) feathers (Figure 2-b) from ornithological specimens containing three bio-pigments: carotenoids (C), psittacofulvins (Ps) and porphyrins (Po) [17,18].

The spectral shape for the carotenoids and psittacofulvins based feathers (both red and yellow) have a sigmoid spectral shape [30,31] and a maximum reflectance of around 80 % for the lighter colors and 20 % for the darker shades. The “spectral location” of the feathers is defined as the wavelength at 50 % of the maximum reflectance and is used to characterized the hue [30,32,33]. In our study, this characteristic wavelength was determined using the first derivative of the reflectance spectra. Indeed, the knee-point of the sigmoid slope on the reflectance spectra coincides with the maximum intensity on the first derivative of the spectra. The positions of the first derivative maxima (λ_m) are

presented in Table 1. Two distinct groups are observed: feathers with a yellow hue (λ_m between 467 and 529 nm) and feathers with a red hue (λ_m between 598 and 639 nm). Some feathers gather in between these two groups with a λ_m between 535 nm and 590 nm. They present various hues of orange, and depending on their ratio of red and yellow bio-pigments a shift of the slope is visible [23,33].

It is difficult to differentiate carotenoids and psittacofulvins bio-pigments based on this spectral characteristic as their λ_m overlap, with both yellow and red feathers. Fortunately, the carotenoid based yellow feathers (Figure 2-a, solid line spectrum) have absorption peaks at approximately 404, 430, 455 and 486 nm [32]; whereas the spectra of the yellow psittacofulvins feathers (Figure 2-a, dotted spectrum) have additional absorption bands around 450 and 475 nm [34]. These absorption bands clearly discriminate yellow carotenoids from yellow psittacofulvins.

Regarding red bio-pigments (Figure 2-b), carotenoids and psittacofulvins have very similar reflectance spectra, and no particular absorption bands are observed. It is thus impossible to discriminate between these two red chemical families using visible reflectance spectroscopy [22]. Porphyrins however (here turacin, see Table 1) present very specific reflectance spectra (Figure 2-b, grey spectrum) with absorption peaks at 452, 544 and 581 nm [22].

Using FORS on feathers can be helpful to determine if the yellow color is due to carotenoids or psittacofulvins. It is however less useful for the most encountered red feathers. One possible solution to identify red bio-pigments would be to probe red feathers that present some orange shades and look for – even weak – spectroscopic features of yellow bio-pigments, as red and yellow colors on a same species are produced by bio-pigments of similar molecular nature [35].

4.2. Microfading tests: Practical and technical considerations

In order to establish an analytical protocol for microfading tests, data were recorded on loose feathers, and most of the presented results were obtained on yellow psittacofulvins-based feathers (PJ04, see Table 1) and yellow carotenoids-based feathers (PJ06, see Table 1) as they were both in sufficient amount to run several experiments.

4.2.1. Feather orientation regarding the measuring head

Feathers have very specific shapes and structures making the microfading testing process complex [36]. To overcome this difficulty, several practical measures were set up. First, it was important to ensure the flatness of the feather barbs, on a local area that corresponds to the size of the light

1 beam, i.e. less than 0,5 mm in diameter. For the Whitmore setup, since the probe is 12 mm away
2 from the sample, light weights were place on the feathers or objects around the area of interest to
3 locally flatten the barbs. For the LED setup, since the probing head is in contact with the sample, light
4 weights were placed on top on the measuring head to ensure a proper contact.

5 Then, the orientation of the barbs regarding the probe was considered. This was pointed out by
6 Riedler *et al.* [16] as being critical: the barbs structure or the measurement geometry can influence
7 the analysis of the color, and hence, the microfading tests. Indeed, the orientation of the barbs can
8 have an impact on the collected data, as their microstructure can provoke the diffraction of the
9 incident light that may influence the reflectance spectra, and thus the measurement of the color
10 change. Riedler *et al.* [16] have come up with a setup using Mylar® templates to position the feather
11 under the probe using different angles on different planes (direction of observation and rotation of
12 the feather regarding the illumination direction and the observation collection). In this work, a
13 normal illumination is used and the direction of the collected light is fixed at 45°. The only angle that
14 varies is the one between the barbs' orientation and the direction of observation. Figure 3-a shows
15 the two positions that were considered: the L position with the barbs in line with the collecting
16 orientation (0° angle), and the P position where the barbs are perpendicular to the collecting
17 geometry (90° angle). For the Whitmore device, the feather was precisely positioned under the
18 probing head by removing the illumination fiber to visualize the analytical spot, and using the crossed
19 lasers to orient the barbs (see Figure 3-b). For the Pesme setup, the Mylar® template from Riedler *et*
20 *al.* [16] was adapted with a thin paper template of the size of the probe, with a hole representing the
21 illumination spot, and two lines representing the L and P positions. The paper is placed in the proper
22 position onto the feather, then removed and replaced by the probing head. Figure 3-c shows the
23 MFT curves that were obtained using the Whitmore setup with the two positions on multiple spots of
24 a yellow psittacofulvins-based feather (PJ04c, see Table 1). The curves obtained in the P position are
25 clearly higher than the ones obtained with the L position, and are close to the BWS4 curve. This is
26 likely due to the contribution of the feather microstructure as it impacts the measured reflectance
27 (and hence the color change) [37]. In order to obtain the most conservative results, the P position
28 must be chosen. Another interesting result is the high variability of the MFT curves regardless the
29 orientation of the feather. This is due to the inherent variability of the pigment concentration across
30 the surface of the feather [28]: in other terms the color saturation varies within a feather leading to
31 high MFT curves (more saturated colors) or lower ones (less saturated ones). In summary, when
32 performing MFT on feathers, three precautions must be followed: the tests must be done with the
33 barbs perpendicular to the measuring geometry, in multiple areas to ensure a homogeneity in the

data (at least 3 tests [20]), and in the most saturated areas where the bio-pigments are more concentrated.

4.2.2. The duration of the tests

Another important parameter is the duration of the microfading tests. Knowing that these tests must be repeated on multiples spots, it would be best from an operational standpoint to perform brief period tests to avoid long analysis campaigns. Indeed, when testing ahead of an exhibition or before answering loan requests, there is often a time constraint: the shorter the tests, the better. Also, the instrument internal drift is a critical parameter that must be considered as it may distort the results. Figure 4-a presents MFT curves on 3 spots of another yellow psittacofulvins-based feather (PJ04I) over a 40 minutes period. The shape of the curve (that will be discussed in paragraph 4.3.1) shows a constant rate starting at 15-20 minutes. The spectrometer seems to be stable on that period of time, as the instrumental drift remains quite flat (Figure 4-a, red dotted line).

4.2.3. The setups and light sources

As mentioned previously, two experimental setups were used in this study, and the main difference is the light source: a xenon lamp versus LEDs (Warm White + Blue at 405 nm). Figure 4-b, shows MFT curves obtained on similar feathers with both devices. For that test, the xenon lamp delivered approximately 0.42 Mlx, whereas the LEDs delivered around 0.01 Mlx (values recorded at the sample). The curves are plotted as function of the time (left) and the dose (right). The consequence on the curves is a rather bad signal to noise ratio for the ones collected with the LEDs system, making the reading of the color change (ΔE^*_{ab}) quite difficult. This result is consistent with the description of the Pesme setup [29] stating that the device is made for testing sample and objects equivalent to BWS1-2-3. Also, the contact mode of the Pesme probe makes the positioning of the feather more difficult than the non-contact Whitmore setup. Whitmore's setup was therefore used for the following MFT experiments.

4.3. Lightfastness of bio-pigments

4.3.1. Shape of the fading rate curves and exposure recommendation

Figure 5 presents the MFT curves that were obtained on the single feathers and the *mqB-JC* loose feathers, in comparison to the BWS curves (BWS2 to BWS5). The presented data are average curves of 3 reiterations with a maximal dose of 0.42 Mlx.h. It must to be noted that the measured doses in our study are higher than usual: a ΔE^*_{ab} of 1.8 is induced on BWS2 after a light dose corresponding of 0.025 Mlux.h and not after the expected light dose of 1 Mlux.h (see below). An explanation would be

that our Whitmore setup includes IR radiation leading to a higher illuminance than usual (xenon peaks between 800 and 1000 nm, see online *supplementary material*), leading to faster color changes. The average values and standard deviations for ΔE^*_{ab} after 40 minutes are also presented (the ΔE_{00} values are available as supplementary materials). The shape of the fading rate curves of organic dyes were described by Cox Crews [38] according to Giles' work [39]. Five types were established, depending on how the dyes are distributed in the sample (diffused, aggregated). Only types I and II will be considered here. Type I presents a fading rate that has a constant decrease, and is attributed to dyes molecularly dispersed throughout the fiber. BWS 1 to 3 fading rate curves match this type [40]. Type II presents an initial rapid rate followed by a slower fading at constant rate. It is supposed to be specific of mostly aggregated colored molecules, with a proportion of exposed dispersed molecules that discolor at the very beginning of the fading process. BWS 4 and 5 fading rate curves are close to this type but present a particularly slow second domain that looks more like a plateau (see Figure 5 and references [40–42]). The fading rate curves for the analyzed feathers (single feathers and from museum objects) are quite similar, and are typical of type II. This seems in agreement with the fact that bio-pigments molecules are incorporated discretely into the shaft, barbs and barbules [16]. Also, there is no particular discrepancy between the tested bio-pigments, in that they all show similar curve shapes. However, the difference in the curve shapes between BWS and bio-pigments may be an issue. Indeed, testing for example psittacofulvins-based feathers for 10 minutes will give BWS4 equivalent, but if the test is longer (20 to 40 minutes) the BWS equivalent shifts to BWS3 or between BWS3 and BWS4 (see Figure 5, yellow curves). This intersection between curves over time was also observed by Tse *et al.* in a study of the light sensitivity of birch bark [43]. Therefore, the minimal duration of the tests should be around 15-20 minutes, and if possible, users must perform one longer test (30-40 minutes) to confirm the kinetics of the color fading.

ΔE^*_{ab} values, after 40 minutes differ depending on the tested bio-pigment: the most sensitive one is the porphyrins-based red feather (ΔE^*_{ab} of 2.2), then the red psittacofulvins-based feathers (ΔE^*_{ab} around 1.5), then the yellow psittacofulvins-based feathers (ΔE^*_{ab} between 0.9 and 1.4) and finally the red and yellow carotenoids-based feathers (ΔE^*_{ab} around 0.8). Beside the nature of the bio-pigment, the difference in the ΔE^*_{ab} can be related to the disparity in the age and conservation conditions of feathers and objects. Indeed, a feather that is newly collected (*i.e.* PR01) will be brighter in color – and richer in bio-pigments – than a feather from an artifact that has already been exposed to light in museums (*i.e.* 71.1917.3.83 D). Also, ritual objects likely start their fading process during ceremonies and dances due to sun exposure [20].

Since, no alternative references to BWS have yet been developed [44], the exposure recommendations are still based on BWS equivalents that categorize the sensitivity of museum

objects. Michalski published the following maximum doses before a JNF (Just Noticeable Fade), in Mlx.h, which is considered as a detection threshold of a perceived color difference, that can be assimilated to the JND: BWS1: 0.3, BWS2: 1, BWS3: 3 and BWS4: 10 with - at 50 lx - a JNF reached in 1.5 to 20 years for BWS1 to 3 respectively; and a JNF in 20 to 700 years for BWS4 to 6 respectively [14]. Ford *et al.* [41] recommend for BWS3-4 equivalents a light level of 50 to 150 lx and an exposure of 5 years per decade for high significance objects. Even if the JND values and associated light doses found in the literature are not really suited for material such as feathers that present more complex color appearance, adopting such values for lighting policy for feathers allows museums to stay on the conservative side. At the *mqB-JC*, based on the concept of "Preservation Target" (PT) defined by Pesme [45], maximum light doses were attributed to Blue Wool Standards Equivalent (BWSE) and a PT set to 1 JND for a hundred years period. Because of the uncertainty of the published figures, and the fact that data has slowly emerged since (see reference [10] and personal communications by C. Pesme on January 15, 2016; and J.-J. Ezrati on December 20, 2016) those doses are the following (Mlx.h): BWSE1: 0.1, BWSE2: 0.7, BWSE3: 3. Concretely, it means that an object containing psittacofulvins-based feathers with a BWS3 equivalent can be presented in the permanent exhibition at 150 lux for a duration of 96 weekly lighting hours for up to four years before a JND. Distinguishing between red porphyrin-based feathers and, at the other end of the sensitivity spectrum, red carotenoids-based feathers, could provide highly valuable insight in deciding the length of exhibition before preparing a replacement object for rotation. At the *mqB-JC*, it has been suggested to set a four years limit at 150 lux for objects containing yellow, green or blue feathers and a two years limit at 100 lux for objects containing red or orange feathers. Hence, should an object contain red or orange porphyrins-based feathers, these set points would enable a rotation before a JND is reached. Admittedly this proposal is on the conservative side, in line with the preservation duties of the museum.

4.3.2. Color changes

In order to demonstrate that the fading is permanent and cumulative, a carotenoids-based feather (PJ06b, see Table 1) was tested in a two-step procedure using the Whitmore setup. A 10 minutes run was performed, then the xenon light was shuttered for 5 min, and finally a second 10 minutes run was achieved, on the same exact spot (the feather was not moved during the whole experiment). Figure 6 shows the two fading curves for both runs. The first run sits above the second one, proving that once the fading has occurred, it is not reversible: the more a feather is exposed, the more it will lose its color.

In order to understand the color changes during the fading procedure, the chromatic coordinates: L^* (brightness), a^* (green–red), b^* (blue–yellow), C^* (saturation) and h^* (hue) were extracted from the reflectance spectra using a homemade software based on the formulas established by the CIE [12] and a D65 reference illuminant and 2° observer. C^* and h^* are calculated using a^* and b^* as:

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad \text{and} \quad h^* = \arctan\left(\frac{b^*}{a^*}\right) \times \frac{180}{\pi}$$

Figure 6 (right side) shows the representation of the chromatic variations in polar scatter charts for both runs. In both cases, the hue does not vary, but the feather shifts to lower C^* values, which means that the yellow color diminishes (loss of saturation). The variation is more important for the first run ($\Delta C_1^*=1.3$) than the second one ($\Delta C_2^*=0.8$).

4.3.3. Near Infrared (NIR) spectral changes

In addition to the visible range, the NIR (1000–2500 nm) was also exploited. This spectral area contains information regarding the molecular structure of the analyzed samples. The aim was to determine if any changes are observable on this spectral area that might be linked to the color change, for example variations in NIR bands relative to the bio-pigments. Figure 7 shows the visible–NIR reflectance spectra that were recorded during a 10 minutes fading test on a psittacofulvins-based feather (PJ04, see Table 1). Most of the bands in the 1200–2300 nm range are assigned to the keratin matrix of the feather, and do not evolve with the time of exposure. The only bands that present a notable decrease with time are the ones at approximately 1935 and 1975 nm (see insert in Figure 7), assigned to water molecules. During the fading process, the intense light beam can induce a local heating of the sample, leading to water loss. After a few hours, the water bands are visible again on the spectra (data not shown here), which means that this process is reversible. This was observed on most of the tested feathers.

5. Conclusions

Three bio-pigments encountered in feathers were studied using Fiber Optics Reflectance Spectroscopy (FORS) and microfading tests (MFT). FORS was able to differentiate yellow carotenoids-based feathers from psittacofulvins-based feathers and to identify red porphyrins-based feathers using the visible range of the spectra. Red carotenoids and psittacofulvins show very similar spectra and cannot be sorted apart. Considering MFT, several practical and technical requirements were set up and discussed. The fading of the bio-pigments is optimal when the feather is at a 90° angle

regarding the geometry of the probing head: indeed, the highest the curve, the more accurate the long-term fading prediction. For similar reasons, analytical spots must be chosen in the most saturated areas of the feathers, where the bio-pigments are in higher concentration. Also, when testing ahead of an exhibition or before answering loan requests, there is often a time constraint. However, the fading tests should be repeated three times to ensure a homogeneity in the data, and last at least 15 to 20 minutes with, if possible, one longer acquisition of 30 to 40 minutes. The loose feathers and the museum objects show similar curves shapes with initial rapid rate followed by a slower fading at constant rate, which is typical of type II. The ΔE^*_{ab} varies between 0.7 and 2.2, which corresponds to a BWSE between 3 and 4. However, the number of tested feathers is not sufficient to draw meaningful conclusions about the lightfastness of the various bio-pigments: additional microfading tests are in process in order to complete this work.

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Figure 1: Top: FORS analysis of carotenoids-based feathers of a specimen of Southern carmine bee-eater *Merops nubicooides* (GuepRose in Table 1). Bottom: loose lumps of feathers from objects of the *mqB-JC* collections that were tested using MFT, from left to right: 70.2004.20.2 (red psittacofulvins-based feather), 70.2004.20.4 (yellow and red psittacofulvins-based feathers), 71.1890.93.158 (red psittacofulvins-based feather) and 71.1917.3.83 D (red carotenoids-based feather).

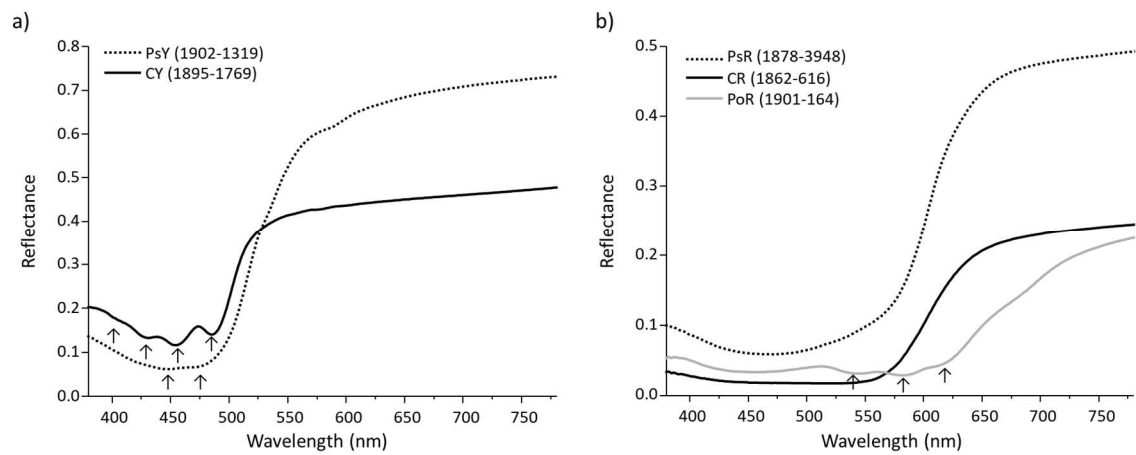


Figure 2: Reflectance spectra obtained on a) yellow (Y) feathers and b) red (R) feathers from ornithological specimens with containing different bio-pigments: carotenoids (C), psittacofulvins (Ps) and porphyrins (Po). The arrows correspond to the absorption bands of the bio-pigments described in the text.

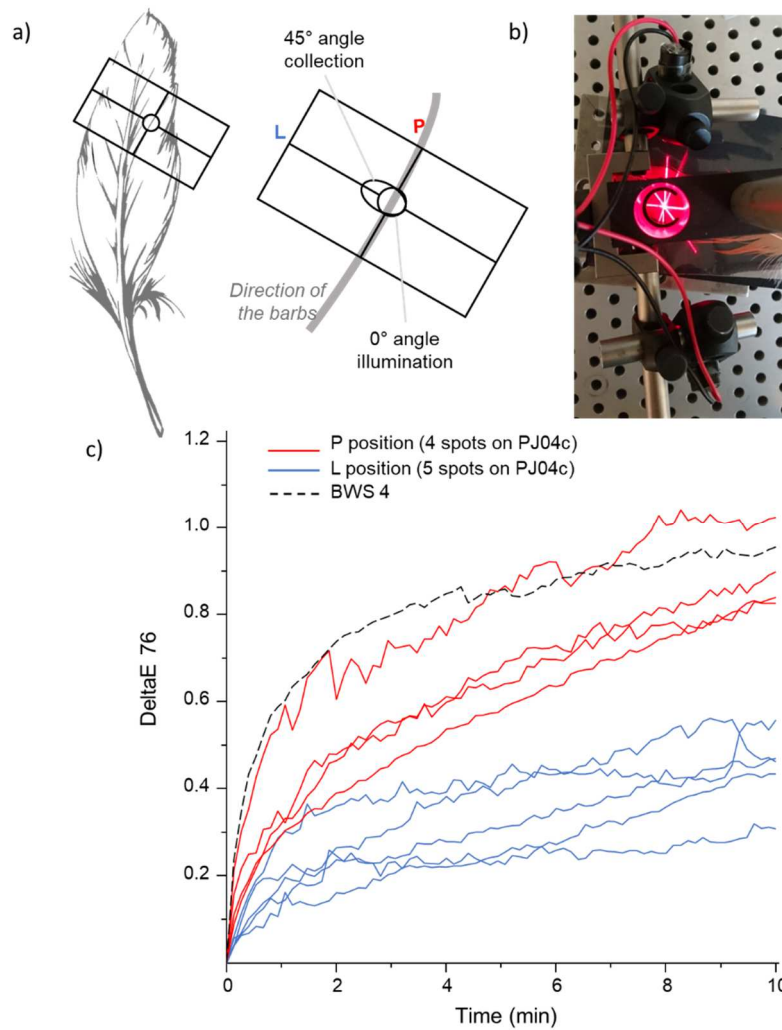


Figure 3: a) Positioning of the feathers regarding the instrumental setup, adapted from [16] ; b) the double crossed lasers for an optimal positionning of the feather; and c) microfading curves on a psittacofulvins-based feather (PJ04c) for both P and L positions, using Whitmore's setup.

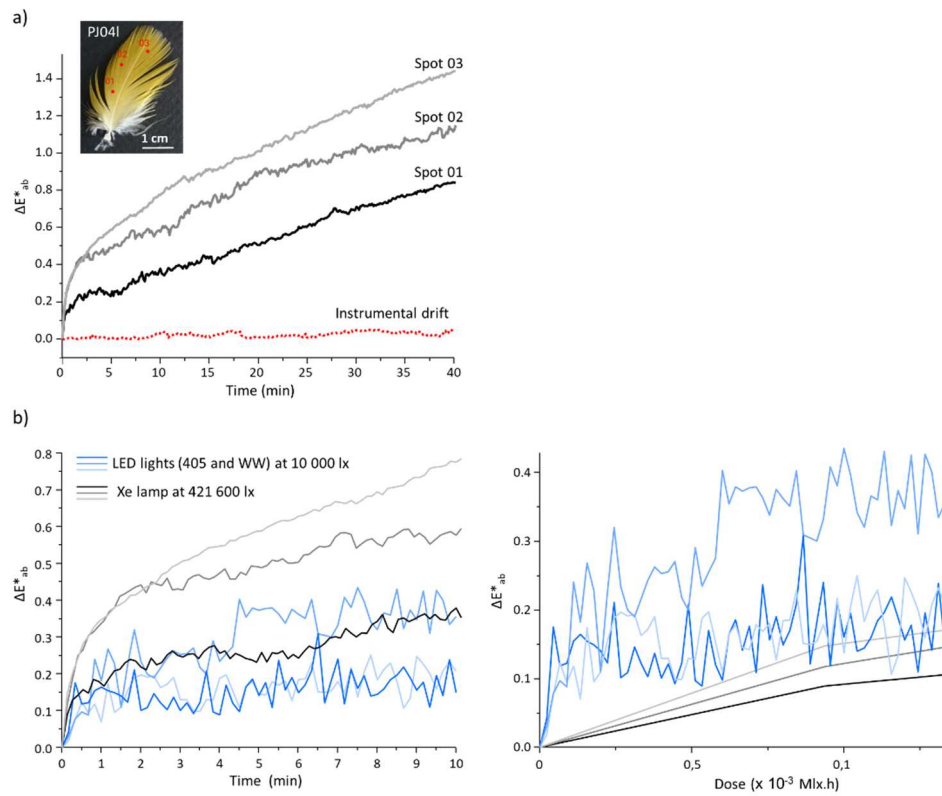


Figure 4: a) Multiple testing of a psittacofulvins-based feather (Blue macaw, *Ara ararauna* - PJ04I) during 40 minutes and comparison with the instrumental drift (Whitmore setup); b) comparison of microfading curves using an xenon lamp (black shades) and LEDs (blue shades), left: as a function of the time, right: as a function of the dose.

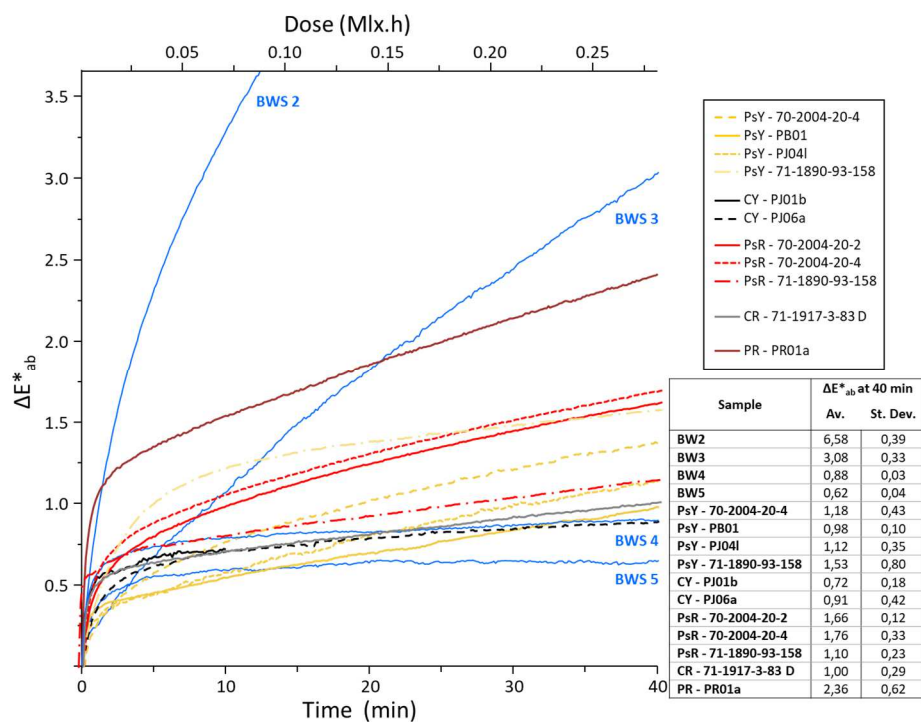


Figure 5: Average microfading curves collected with the Whitmore setup for 40 minutes on psittacofulvins-based (Ps), carotenoids-based (C) and porphyrins-based (P) feathers a) yellow (Y) and red (R) loose feathers and b) on some feathers from the *mqB-JC* objects. The dose is presented on the top axis, and data are compared to the BWS2 to 5. The table presents the average values and standard deviations for ΔE^*_{ab} after 40 minutes (the ΔE_{00} values are available as supplementary materials).

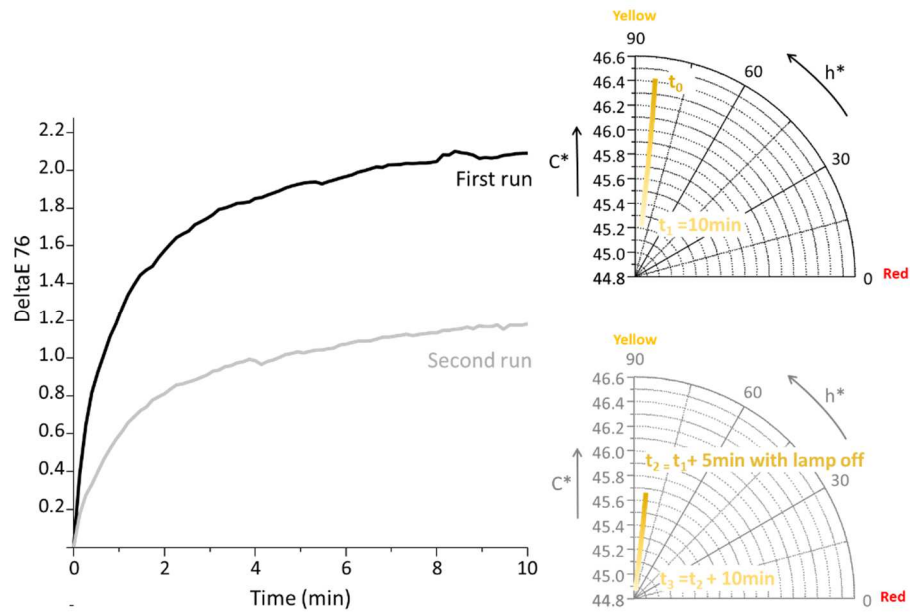
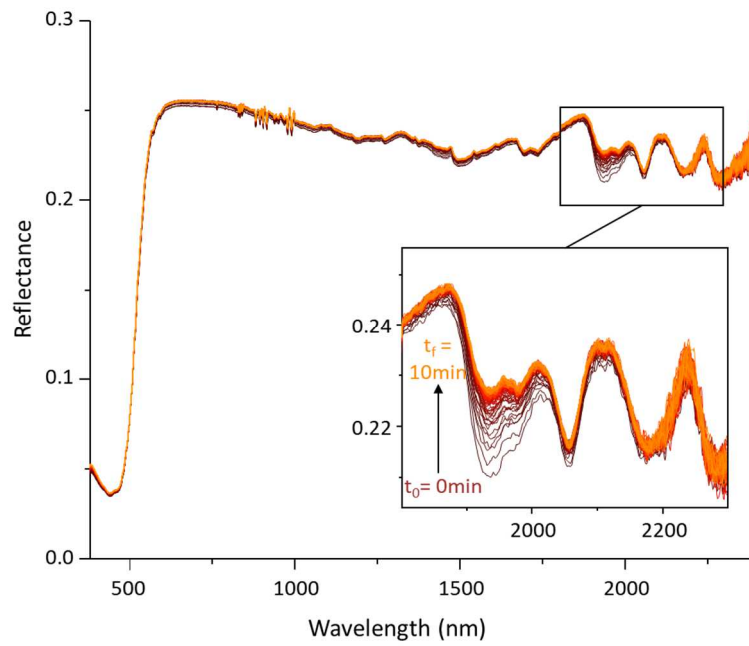


Figure 6: Color change experiment (see text) on a carotenoids-based yellow feather (PJ06): microfading curves and representation of the chromatic variations in polar scatter charts for both runs.

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Figure 7: visible-NIR reflectance spectra recorded during a 10 minutes fading test on a psittacofulvins-based feather (PJ04). An enlarged view of the 1800-2300 nm region is presented in the insert.

Collection	Sample or accession number	Origin and date	Species of the analyzed feather(s)	Analyzed color(s) of the feather(s)	Biopigment	λ_m (maximum of the 1 st derivative of the FORS spectra)	MFT
Loose feathers	PJ01	Unknown origin Unknown date	<i>Emberiza citrinella</i>	Yellow	Carotenoids (lutein, zeaxanthine)	498	X
	PJ03	Unknown origin Unknown date	<i>Carduelis chloris</i>	Yellow	Carotenoids (lutein, canary xanthophyll A, canary xanthophyll B)	/	X
	PJ04	Brazil 1960-65	<i>Ara ararauna</i>	Yellow	Psittacofulvins	515	X
	PJ05	Unknown origin Unknown date	<i>Icterus atrogularis</i>	Yellow	Carotenoids	/	X
	PJ06	Birmingham (USA) 1993	<i>Icterus galbula</i>	Yellow/orange	Carotenoids (lutein, canary xanthophyll A, canary xanthophyll B, astaxanthine, alpha-doradexanthine, canthaxanthine, echinenone)	506	X
	PJ07	Unknown origin 1985	<i>Regulus regulus</i>	Yellow	Carotenoids (lutein, zeaxanthine)	/	X
	PJ08	Unknown origin 1995	<i>Oriolus oriolus</i>	Yellow	Carotenoids (lutein, zeaxanthine)	500	X
	PJ09	Unknown origin Unknown date	<i>Motacilla (flava) feldegg</i>	Yellow	Carotenoids (lutein, zeaxanthine)	505	
	PJ10	Unknown origin 1951	<i>Oriolus oriolus</i>	Yellow	Carotenoids (lutein, zeaxanthine)	505	
	PJ11	Pakistan 1996	<i>Emberiza melanocephala</i>	Yellow	Carotenoids (lutein, zeaxanthine)	505	
	PG01	Paris menagerie (feral) Unknown date	<i>Psittacula krameri</i>	Yellow	Psittacofulvins	505	X
	PR01	Paris menagerie 2018	<i>Tauraco cf. leucotis</i>	Red	Porphyrins (turacin = copper complex of uroporphyrin)	603	X
	PB01	Paris menagerie 2018	<i>Ara ararauna</i>	Yellow	Psittacofulvins	511	X
Ornithological specimens	1847-1340	Brazil 1847	<i>Pitangus sulphuratus</i>	Yellow	Carotenoids	495	
	1858-1762	Southern Africa 1858	<i>Macronyx capensis</i>	Yellow / orange	Carotenoids	557	
	1862-616	Unknown origin 1862	<i>Ramphastos culminatus</i>	Yellow Red	Carotenoids	523 602	

Collection	Sample or accession number	Origin and date	Species of the analyzed feather(s)	Analyzed color(s) of the feather(s)	Biopigment	λ_m (maximum of the 1 st derivative of the FORS spectra)	MFT
	1864-284	Mexico 1864	<i>Icterus gularis</i>	Yellow	Carotenoids	535	
	1877-1966	Equator 1877	<i>Anisognathus somptuosus</i> spp.	Yellow	Carotenoids	507	
	1878-362	Chandannagar (India) 1878	<i>Oriolus kundoo</i>	Yellow	Carotenoids	513	
	1878-3948	Guatemala 1878	<i>Amazona guatemalae</i>	Red/orange	Psittacofulvins	603	
	1894-361	Brazil 1894	<i>Ramphastos Ariel</i>	Yellow Red	Carotenoids	519 582	
	1895-1769	Central Asia 1895	<i>Motacilla citreola calcarata</i>	Yellow	Carotenoids	503	
	1897-32	Unknown origin 1896	<i>Eos histrio talautensis</i>	Red/orange	Psittacofulvins	604	
	1901-164	East Africa 1900	<i>Tauraco leucotis</i>	Dark red/purple	Porphyrins (turacin = copper complex of uroporphyrin)	639	
	1902-1319	Guyana 1902	<i>Chrysotis amazonicus</i>	Yellow Orange/red	Psittacofulvins	516 600	
	1902-1344	Guyana 1902	<i>Cassiculus melanicterus</i>	Yellow	Carotenoids	509	
	2003-80	Mulhouse Zoo (France) 2003	<i>Amazona vinacea</i>	Red	Psittacofulvins	602	
	4111	New Guinea Before 1900	<i>Dasyptilus pesqueti</i>	Red	Psittacofulvins	606	
	6000	Mexico 1843	<i>Cassiculus melanicterus</i>	Yellow	Carotenoids	502	
	GuepRose	East Africa (German part) Before 1900	<i>Merops nubicoides</i>	Pink	Carotenoids	588	
	N362-VS4	Unknown origin Unknown date	<i>Merops nubicoides</i>	Red/pink	Carotenoids	569	
	Paroaria	Brazil Unknown date	<i>Paroaria capitata</i>	Red	Carotenoids	589	
Archaeological	70.2002.9.1	Peru	<i>Ara ararauna</i>	Yellow	Psittacofulvins	516	

Collection	Sample or accession number	Origin and date	Species of the analyzed feather(s)	Analyzed color(s) of the feather(s)	Biopigment	λ_m (maximum of the 1 st derivative of the FORS spectra)	MFT
and ethnographic objects	Feather tunic	700 - 1100					
	70.2003.2.2 Woven mat	Guyana/Brazil 20th C.	<i>Psarocolius sp.</i> <i>Ara chloroptera</i>	Yellow Red	Carotenoids Psittacofulvins	508 605	
	70.2004.20.2 Poncho	Peru 1400-1500	<i>Ara ararauna</i> <i>Ara macao</i>	Yellow Red	Psittacofulvins	520 600	X (red)
	70.2004.20.4 Feather tunic	Peru 1000 - 1450	<i>Ara ararauna</i> <i>Ara chloroptera</i>	Yellow Red	Psittacofulvins	514 604	X
	70.2008.41.29 Hair ornament	Brazil 1960-1972	<i>Ara macao</i>	Yellow Red	Psittacofulvins	514 598	
	70.2008.41.31.1-2 Earrings	Brazil 1960-1972	<i>Ramphastos toco</i>	Yellow Red	Carotenoids	518 612	
	70.2008.41.33.1-2 Earrings	Brazil 1960-1972	<i>Ramphastos toco</i>	Yellow Red	Carotenoids	502 569	
	70.2008.41.35.1-2 Earrings	Brazil 1960-1972	<i>Ara ararauna</i> <i>Ramphastos toco</i>	Yellow Red	Psittacofulvins Carotenoids	516 605	
	70.2008.41.36.1-2 Earrings	Brazil 1960-1972	<i>Ramphastos toco</i>	Yellow Red	Carotenoids	467 604	
	70.2008.41.50.8 Headdress-mask	Brazil 1960-1972	<i>Amazona aestiva</i>	Red	Psittacofulvins	601	
	70.2008.41.50.9 Headdress-mask	Brazil 1960-1972	<i>Ara macao</i>	Yellow Red	Psittacofulvins	509 605	
	70.2008.41.89 Headdress	Brazil 1960-1972	<i>Ara chloroptera</i>	Red	Psittacofulvins	604	
	70.2008.41.90.1-2 Earrings	Brazil 1960-1972	<i>Ara macao</i>	Yellow Red	Psittacofulvins	540 603	
	70.2008.41.91.1-2 Earrings	Brazil 1960-1972	<i>Xipholena punicea</i>	Purple (red + structural blue)	Carotenoids	605	
	70.2008.41.101 Child's shoulder strap	Brazil 1960-1972	<i>Ara macao</i>	Red	Psittacofulvins	607	
	70.2008.41.106.1-2 Earrings	Brazil 1960-1972	<i>Ara chloroptera</i>	Red	Psittacofulvins	606	
	70.2008.41.114 Headdress	Brazil 1960-1972	<i>Ara macao</i> <i>Psarocolius decumanus</i>	Yellow Red	Psittacofulvins Carotenoids	510 601	
	70.2008.41.115	Brazil	<i>Ara macao</i>	Yellow	Psittacofulvins	509	

Collection	Sample or accession number	Origin and date	Species of the analyzed feather(s)	Analyzed color(s) of the feather(s)	Biopigment	λ_m (maximum of the 1 st derivative of the FORS spectra)	MFT
	Headdress	1960-1972	<i>Psarocolius decumanus</i>	Red	Carotenoids	599	
	70.2008.41.116 Headdress	Brazil 1960-1972	<i>Ara macao</i> <i>Psarocolius viridis</i>	Yellow Red	Psittacofulvins Carotenoids	509 602	
	70.2008.41.135 Diadem	Brazil 1960-1972	<i>Psarocolius viridis</i>	Yellow	Carotenoids	506	
	70.2008.41.142 Diadem	Brazil 1960-1972	<i>Ara macao</i> <i>Psarocolius decumanus</i>	Yellow Red	Carotenoids Psittacofulvins	509 604	
	70.2008.41.147 Headdress	Brazil 1960-1972	<i>Ara macao</i> <i>Ara macao</i> <i>Ara chloroptera</i>	Yellow Red Red	Psittacofulvins	508 600 602	
	70.2008.41.157 Diadem	Brazil 1960-1972	<i>Ara macao</i>	Yellow Red	Psittacofulvins	514 601	
	70.2008.41.159 Diadem	Brazil 1960-1972	<i>Ara macao</i>	Red	Psittacofulvins	604	
	70.2008.41.163 Back pendant	Brazil 1960-1972	<i>Ara ararauna</i> <i>Ara macao</i>	Yellow Red	Psittacofulvins	517 605	
	70.2008.41.164 Back pendant	Brazil 1960-1972	<i>Ara chloroptera</i>	Red	Psittacofulvins	607	
	70.2008.41.165 Back pendant	Brazil 1960-1972	<i>Ara macao</i> or <i>Amazona sp. or Pionites sp.</i>	Red	Psittacofulvins	604	
	70.2008.41.171 Necklace	Brazil 1960-1972	<i>Ara chloroptera</i>	Red	Psittacofulvins	605	
	70.2008.41.179 Headdress	Brazil 1960-1972	<i>Amazona aestiva</i>	Red	Psittacofulvins	602	
	70.2008.41.192 Armlet	Brazil 1960-1972	<i>Ara ararauna</i> <i>Ara chloroptera</i>	Yellow Red	Psittacofulvins	541 607	
	70.2008.41.193 Armlet	Brazil 1960-1972	<i>Ara ararauna</i> <i>Ara chloroptera</i>	Yellow Red	Psittacofulvins	543 605	
	70.2009.49.1 Woven mat	Brazil Late 20th c.	<i>Ara ararauna</i> <i>Ara macao</i>	Yellow Red	Psittacofulvins	512 601	
	70.2010.1.18 Crown	Brazil 1960-1972	<i>Ara ararauna</i> <i>Ara chloroptera</i>	Yellow Red	Psittacofulvins	540 604	
	70.2010.1.32.1-2	Brazil	<i>Ara macao</i>	Red	Psittacofulvins	602	

Collection	Sample or accession number	Origin and date	Species of the analyzed feather(s)	Analyzed color(s) of the feather(s)	Biopigment	λ_m (maximum of the 1 st derivative of the FORS spectra)	MFT
	Nose ornament	1960-1972					
	70.2010.1.53 Bracelet	Brazil 1960-1972	<i>Ara macao</i>	Yellow Red	Psittacofulvins	507 601	
	70.2010.1.86.1-2 Pair of armlets	Brazil 1960-1972	<i>Ara ararauna</i> <i>Ara macao</i>	Yellow Red	Psittacofulvins	511 608	
	70.2010.1.87.1-2 Pair of armlets	Brazil 1960-1972	<i>Ara ararauna</i> <i>Ara macao</i>	Yellow Red	Psittacofulvins	510 604	
	71.1881.34.109 Bracelet	Colombia 1850 - 1879	<i>Xipholina punicea</i>	Purple (Red + structural blue)	Carotenoids	602	
	71.1881.34.130 Feather crown	French Guyana 1850 - 1879	<i>Ramphastos tucanus</i>	Yellow Red	Carotenoids	519 590	
	71.1890.93.158 Feather headdress	French Guyana 1880-1885	<i>Ara macao</i> <i>Ara ararauna</i>	Red Yellow	Psittacofulvins	601 /	x
	71.1939.25.335 Woven mat	French Guyana 1900 - 1938	<i>Ara macao</i>	Red	Psittacofulvins	605	
	71.1939.88.543 Nose pin	Brazil Unknown date	<i>Ara macao</i>	Red	Psittacofulvins	602	
	71.1939.88.544 Nose pin	Brazil Unknown date	<i>Ara macao</i>	Red	Psittacofulvins	601	
	71.1917.3.83 D Tupinamba cape	Brazil 16th c.	<i>Eudocimus ruber</i>	Red	Carotenoids	/	x

Table 1: Studied samples, ornithological specimens and museum objects along with the bird species they come from, their origins, their colors and the biopigments they contain [17,18]. The analyses that were performed for each sample or object are also indicated, with the wavelength corresponding to the feathers color (calculated from the first derivative of the reflectance spectra, see text for more information). The “/” sign indicates that the FORS analysis was not performed due to a restricted access to some feathers.