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We review the state of the art in the research on the fluorescence emitted by plant leaves, fruits, flowers, avian, butterflies, beetles, dragonflies, millipedes, cockroaches, bees, spiders, scorpions and sea organisms and discuss its relevance in nature.

Reviewing the relevance of fluorescence in biological systems

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Abstract

Fluorescence is emitted by diverse living organisms. The analysis and interpretation of these signals may give information about their physiological state, ways of communication among species and presence of specific chemicals. In this manuscript we review the state of the art in the research on the fluorescence emitted by plant leaves, fruits, flowers, avian, butterflies, beetles, dragonflies, millipedes, cockroaches, bees, spiders, scorpions and sea organisms and discuss its relevance in nature.

Introduction

Luminescence, from the latin *lumen* (light), is "a spontaneous emission of radiation from an electronic excited species (or from a vibrationally excited species) not in thermal equilibrium with its environment".[1] Under the term "luminescence" different processes are encompassed: fluorescence, phosphorescence, chemiluminescence, bioluminescence, electroluminescence, cathodoluminescence and radioluminescence.[2] Many of these processes are important in nature but in this review we will only refer to fluorescence, which is the emission of light resulting from the electronic transition between states with the same spin multiplicity following excitation by energy absorption.

The first observation and report of fluorescence from natural systems was connected to the finding by the Spanish of new medicinal herbs that came to Europe from West Indies in the XVI century. In fact, not only gold, silver and precious stones were taken from America to Europe, but also animals and valued plants with healing qualities (Figure 1).[3] In 1565, Nicolás Monardes, a Spanish physician and botanist observed and reported a peculiar blue colour (Monardes did not know at that moment it was blue fluorescence) in infusions of a particular type of wood from Mexico (*Lignum nephriticum*):

> *"Toman el palo y hacen unas tajaditas muy delgadas y no muy grandes y échanlas en agua…dentro de media hora se comienza el agua a poner con un color azul muy claro y cuánto más va más azul se torna…"*3

that means:

" They take the wood and make slices of it as thin as possible and not very large and place them in water...Half an hour after the wood was put in, the water begins to take a very pale blue colour, and it becomes bluer the longer it stays… "

Figure 1

Figure 1. Introductory fragment from the book written by Nicolás Monardes in 1565: Historia medicinal de las cosas que se traen de nuestras Indias Occidentales que sirven en Medicina, Primera parte.

Monardes used this infusion to treat kidney and urinary diseases. Aztecs already used and knew about the beneficial effects of this tea prepared from the wood of the species *Eysenhardtia polystachya*.[4,5]

Interestingly, the book written by Monardes also contained the first reported use of fluorescence as an indicator of drugs quality:

> *"El palo de la orina ha de hacer el agua "azul" y advierta que este palo que llamo de la orina y ijada, haga el agua azul, porque si no la hace azul no es lo verdadero porque traen ahora un palo que hace al agua amarilla y éste no es el que aprovecha, sino que haga el agua azul porque el tal que la hiciere azul será el verdadero"*.3

which briefly remarks: "The water with the wood for kidney disease should take a blue colour. If it takes a yellow colour the wood is not legitimate."

The Franciscan Friar Bernardino de Sahagún (1499 -1590) also referred to this wood as "*coatli*" in the Florentine codex.[6] The fluorophore responsible for the blue fluorescence of the infusions above mentioned was called matlaline (Table 1) (from

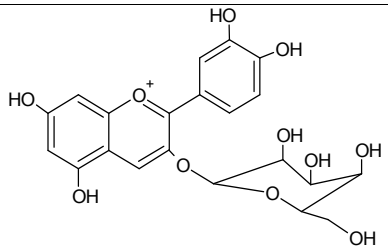
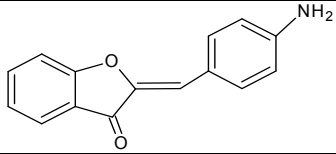
Matlali, the Aztec word for blue) with an emission maximum around 466 nm and with a high fluorescence quantum yield value (close to 1).[7]

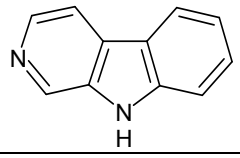
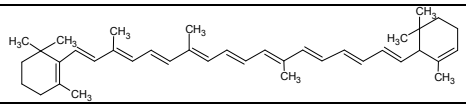
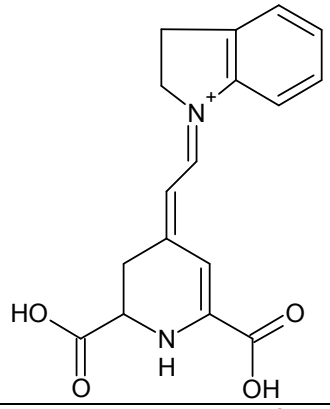
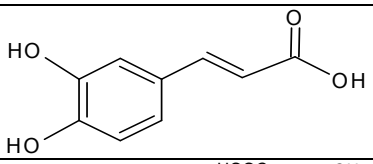
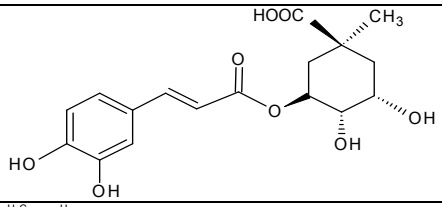
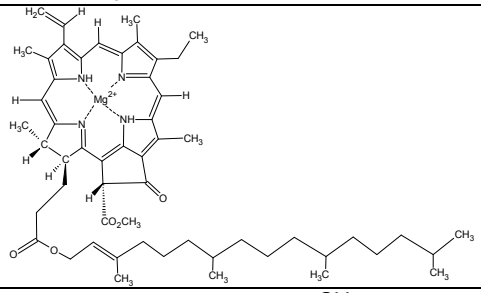
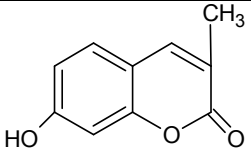
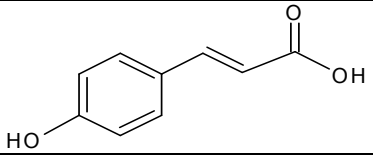
A.

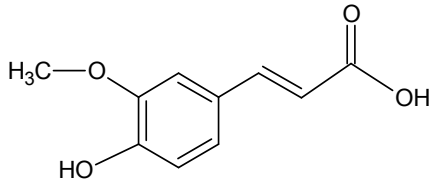
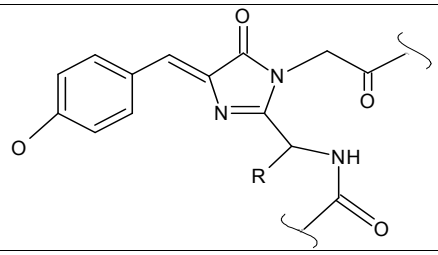
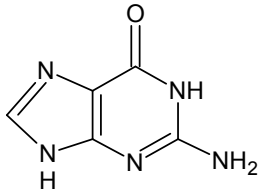
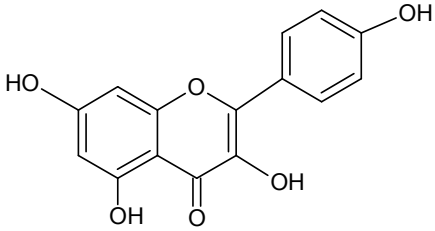
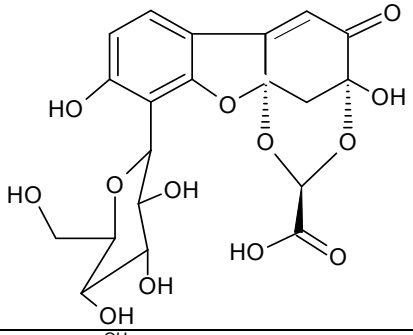
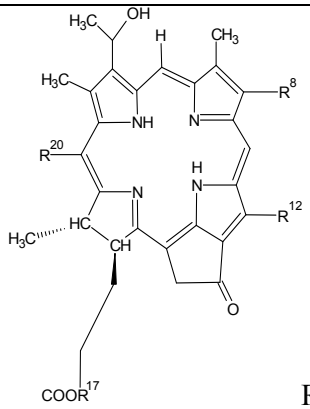
	Biological system	Fluorescent compounds
Plants	Wood and bark	Matlaline[3-7] Quinine[10]
	Leaves	Chlorophyll-a[13-19] Rosmarinic acid[37] Ferulic acid[36,37] p-Coumaric[37] Chlorogenic acid[37] Caffeic acid[37] Kaempferol[45] Quercetin[43]
	Fruits	Chlorophyll-a[55-68] Ferulic acid[69] Lipofuscin[70]
	Flowers	Chlorophyll-a[73] Rosmarinic acid[88] Ferulic acid[88] p-coumaric[88] Chlorogenic acid[88] Caffeic acid[88] Betaxanthins[75-82] Betacyanins[75-82] Aurones[83,84] Anthocyanins[73,85,88,89] Beta Carotene[73,92] Rhodopin[92] Spheroidenone[92]

Animals	Avians	Psittacofulvins[94-95] Carotenoids[94] Spheniscin[110-111]
	Butterflies and moths	Pterins[116]
	Beetles	Pterins[124-127]
	Dragonflies	Pterins[129] Resilin[129]
	Millipedes	Pterins[131]
	Cockroaches	Lipofuscin[132]
	Bees	Schiff-bases[134]
	Spiders and scorpions	Beta Carboline[137] Coumarine derivative[138]
	Sea organisms	GFP[142-144] Porphyrins[148] Cyan proteins[170] Yellow proteins[145] Red proteins[145] Guanine[172] Pheophorbide-a[175]

B.

Fluorophore name or family	Structure	$\lambda_{abs}/\lambda_{em}$ (nm)	References
Anthocyanins (Cyaniding-3-glucoside)		527/600 (methanol) 533/624 (intact petals of *Rodhodendrum indicum*)	88
Aurones (4′-Aminoaurone)		430/560 (water)	84

Beta-carboline		375/450 (ethanol-water mixture)	137
Beta-caroteno		480/580 (CS_2)	92
Betacyanin		524/570 (water)	82
Caffeic acid		206, 281 and 310/432 (methanol)	188, 44, 42
Chlorogenic acid		330/440 (methanol)	41, 42
Chlorophyll-a		480 and 680/680-690 and 730-740 (intact leaves)	21, 32
4-methyl-7-hidroxy-Coumarin		410/440 (ethanol-water mixture)	138
p-Coumaric acid		280/415-445 (methanol)	37, 40

Ferulic acid		240 and 340/ 400-480 (Solvent dependence)	39
Green Fluorescence Protein		395 and 470/509 and 540	189, 142
Guanine		500-570/584-699	190, 172
Kaempferol		260-270 and 360-380/520 (diphenylboric acid-2-aminoethyl ester)	37, 45
Lipofuscin	Unknown structure	360-380/440-470	70
Matlaline		307 and 382 (pH=4-5,5), 283 and 430 (pH=9)/466 (water solution)	191, 7
Pheophorbides	 R^8 is Et, Pr^n, Bu^i or Pn^{neo}; R^{12} is Me or Et; R^{20} is Me or H and R^{17} is Me.	400/670 (unpurified retinal cell suspension in 20% sucrose in PIPES-buffered saline)	175

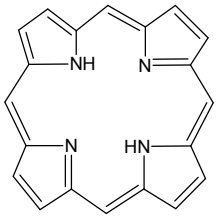
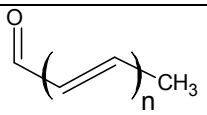
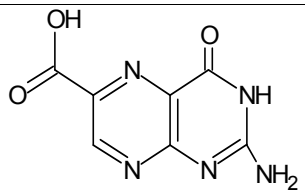
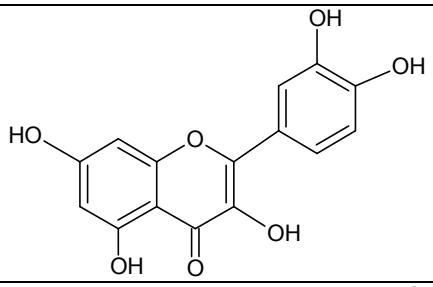
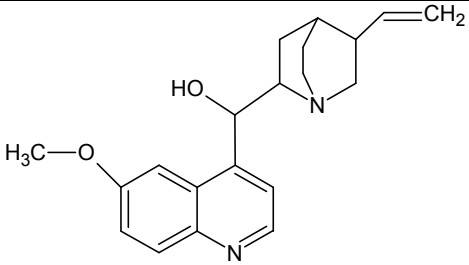
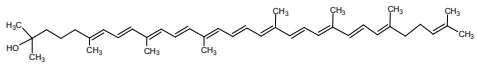
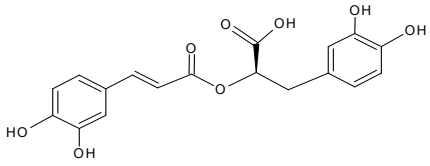
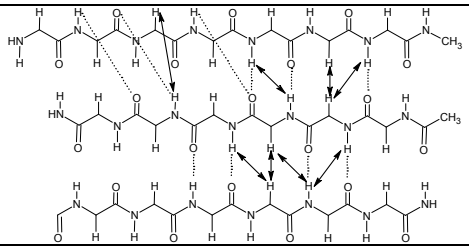
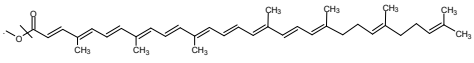
Porphyrins derivative		410/620 (purified mature photophore extract)	148
Psittacofulvin	 n= 6 - 9	420-450/527	93,98
Pterins (Pterin-6-carboxilic acid)		350/450 (methanol)	131
Quercetin		250 and 370 (methanol)/ 500-540 (cellular milieu)	192, 43
Quinine		347/450 (0.5M H_2SO_4)	11, 187
Rhodopin		500-550/560-600	92
Rosmarinic acid		260-380/440-450 (methanol-water mixture at pH 7)	38
Spheniscin		370-400/450-500 (aqueous alkaline solution)	110, 111
Spheroidenone		520/570-610 (CS_2)	92

Table 1. **A.** Common fluorophores found in biological organisms. **B**. Chemical structure and optical properties for natural fluorophores.

A few years ago, it was found that this compound matlaline is not present in the plant but it results from an oxidation of flavonoids.[7]

From this first observation up to 1852 the phenomenon of fluorescence was vaguely described using non-specific terms as reflectance or dispersion. In 1833, David Brewster detected chlorophyll fluorescence. He described the process indicating that when a beam of sun light passed through an alcohol solution of leaves, a red beam could be observed. Remarkably, he also noticed that "*By making the ray pass through greater thickness in succession, it became first orange and then yellow and yellowish-green"* first reporting fluorescence re-absorption processes in concentrated solutions.[8,9]

Another fluorescent natural compound known from ancient times is quinine (Table 1). This is an alkaloid with medicinal properties that is found naturally in the bark of *Cinchona* tree, a species native to the tropical Andes in the western South America. *Cinchona* barks were exported by the Jesuits to Rome in the beginning of the 17th century.[10] In 1845, Herschell, reported fluorescence from quinine solutions referring to this emission as "epipolic dispersion" due to the observation of a superficial colour (from the greek *epipole* = surface).[11]

In the 19th century, Stokes also studied the quinine emission performing an experiment in which he used a prism to disperse sunlight. When he placed a test tube with quinine solution beyond the blue portion of the spectrum (UV), he observed a blue emission *"*having an unearthly appearance*"*. Initially, he used the term "dispersive reflection" to describe it but unsatisfied with this name; he introduced for the first time

the term fluorescence (from fluorspar, a mineral that displayed blue light emission when irradiated in the UV).[12]

As it can therefore be seen, fluorescence from compounds naturally present in biological systems (especially plants) has been strongly linked to the history of this photophysical process. Every year, new evidence of emitting biological tissues in animals and plants appears and great interest in understanding the origin and function of this luminescence thoroughly arises. An important point is unveiling whether naturally-occurring fluorescence acts as a biosignal or it is simply a non-functional consequence due to either the chemical structure of the pigments or the presence of nanostructures in the tissue. To address this dilemma, several researchers have undertaken spectroscopic, microscopic and modeling studies while others have worked directly on behavioural experiments on animals. The wide varieties of experiments that have been conducted, have tried to contribute to the understanding of the origin and role of fluorescence in nature. In this manuscript we attempt to review and summarize the main findings in this fascinating area of photochemistry.

Fluorescence in Biological Systems

Plants

Leaves

Plant leaves fluoresce in the blue, green, red and far-red region of the electromagnetic spectrum.

Red and far-red fluorescence is due to the emission of chlorophyll-a contained in the chloroplasts (Table 1).[13] In plants, the major part of the light absorbed by the leaves (more than 80%) is used in the process of photosynthesis, a small portion of the

absorbed radiation is dissipated as heat and another small portion (less than 2%) is emitted as fluorescence, being these three processes in competition.[14,15]

Figure 2

Figure 2. Chlorophyll-a emits red and far red fluorescence *in vivo* under UV or blue excitation.

In1931, a paper from Kautsky and Hirsch revolutionized the knowledge in the research of chlorophyll fluorescence. In fact, their work titled "New experiments on carbon dioxide assimilation" correlated the chlorophyll fluorescence of dark-adapted leaves, with carbon dioxide assimilation.[16] These observations were a successful starting point in the connection between chlorophyll fluorescence and photosynthesis giving place to a high number of works in this field since then.[13] An amazing feature of the chlorophyll fluorescence in photosynthetic organisms is their change over time. In fact, when chlorophyll-a in the reaction center of photosystem II (PSII) is excited, it transfers electrons to the primary acceptor quinone Q_A. Once Q_A has accepted an electron, it cannot accept another until it has been transferred to the next acceptor Q_B. During this time, the reaction center is described as “closed” and the fluorescence emission increases from an initial value F_0 up to a maximum value F_m (Figure 3). Then, fluorescence starts to fall in a process called “fluorescence quenching” to finally reach a stationary state (F_s). The fluorescence quenching has a photochemical and a non-photochemical contribution. The photochemical quenching (q_p) is due to activation of enzymes involved in the carbon metabolism induced by light and the opening of stomata. The non-photochemical quenching (q_{Np}) is due to an increase in the yield of

heat dissipation.[17] The whole variable process is usually called Kautsky kinetics and information about the photosynthetic process may be inferred from it.[18]

Figure 3

Figure 3. Variable chlorophyll fluorescence recorded with a pulse-modulated fluorometer for a typical plant leaf. Reproduced from reference 18. For a detailed description of this process see references 17-19.

From Kautsky kinetics the maximum quantum yield of PSII: $(F_m-F_0)/F_m = F_v/F_m$, from dark adapted leaves and the effective PSII quantum efficiency for light adapted leaves: $(F'_m-F_s)/F'_m$, (where F'_m is the maximum fluorescence for light adapted leaves) may be obtained.[18,19]

If a plant leaf is excited with a low photon flux (lower than 20 $\mu mol.m^{-2}.s^{-1}$), variable fluorescence is not induced and a constant spectral distribution characterized by two bands: one in the red region around 680 nm, from PSII, and the other in the far-red at about 735 nm, from both PSII and PSI, is obtained (Figure 4).[20,21,22]

Figure 4

Figure 4. Absorption spectrum (thin line) and F_0 fluorescence emission spectrum corrected by the detector response to wavelengths (thick line) for a leaf of *Ficus benjamina*. Excitation wavelength: 460 nm.

The fluorescence ratio $F_{red}/F_{far-red}$ has been correlated with environmental stress conditions and chlorophyll content in plants.[23,24,25,26]

According to Buschmann, the fluorescence ratio of leaves decreases with increasing chlorophyll concentration due to re-absorption processes that affect mainly the red band.[14]

Other authors support that this ratio depends not only on the chlorophyll concentration but also on the particular contribution of each photosystem to the fluorescence.[21,27,28]

The observed chlorophyll fluorescence from intact leaves is usually affected by light re-absorption processes. In fact, whenever there is an overlap between the absorption and emission spectra, the observed fluorescence spectra are distorted.[29] In plants, the experimental fluorescence spectrum from intact leaves differs from the true spectrum originating within chloroplasts. The physiological state of a plant is strictly related to the true spectrum and not to the experimental one and it is relevant to derive the original spectrum from the observed one. Several correction models for this purpose have been proposed in literature.[30,31,32]. A detailed comparison and analysis of these models, which lead to different results, was performed by Cordon and Lagorio.[33]

Some authors suggested that after correction to eliminate re-absorption artifacts, the fluorescence ratio could be connected with either the content of PSII relative to PSI or the disconnection between both photosystems.[34,35] For instance, in shaded leaves as in the abaxial part of leaves, the corrected fluorescence ratio resulted higher than for sunlight leaves and adaxial parts of leaves. This result was interpreted in terms of a higher proportion of PSII relative to PSI developed in the leaf grown under far-red-rich light which favors PSI absorption.[34]

Upon UV excitation, blue and green fluorescence is also observed for leaves. It is reported that the main responsible for this emission are hydroxycinnamic acids. In particular, most of this fluorescence comes from ferulic acid covalently linked to

polysaccharides in the cell wall of leaves epidermis.[36,37] Actually, phenolic acids as rosmarinic, ferulic, p-coumaric, chlorogenic and caffeic acids, and flavonoids as quercetin and kaempferol have been reported as fluorescent compounds in plants (see Table 1). Rosmarinic acid in methanol-water mixture at pH 7 presents an emission maximum in the blue at 440-450 nm,[38] ferulic acid displays a solvent dependent emission maxima in the region from 400 to 480 nm,[39] p-coumaric acid fluoresces in the range from 415 to 445 nm,[40] chlorogenic acids in methanol fluoresces around 440 nm[41] and caffeic acid at 432 nm.[42] Regarding quercetin, Nifli *et al.* has reported its fluorescence in cellular milieu from 500 to 540 nm.[43] However, some authors speculated it could be extremely weak due to the observation of the Raman band in the published spectrum.[44] Kaempferol was also reported to emit in the green around 520 nm, but this fluorescence was induced by diphenylboric acid-2-aminoethyl ester.[45]

The fluorescence ratios $F_{blue\text{-}green}/F_{red}$ or $F_{blue\text{-}green}/F_{far\text{-}red}$ are usually affected by stress factors.[46] The fluorescence ratio $F_{blue\text{-}green}/F_{red}$ may be distorted by light re-absorption processes.[47,48,49] As far-red fluorescence is usually no affected by re-absorption, the fluorescence ratio $F_{blue\text{-}green}/F_{far\text{-}red}$ is preferred than $F_{blue\text{-}green}/F_{red}$ when correlations with stress factors are looked for.

Plants fluorescence has become extremely relevant as a tool for obtaining nondestructively information about photosynthesis, plant physiology as on stress and pollutant effects on plants. [39,50,51,52] Additionally, it has been recently used in quality assessment and quantification of nutraceutics.[44] Plant fluorescence is an extense topic and what we have presented above is only a brief sample of the numerous works in this area.

Fruits

Many fruits contain chlorophyll in varying amounts during their growth, harvesting and post harvesting periods. Fruits containing chlorophyll perform photosynthesis and they display also variable fluorescence similar to leaves. An excellent review about fruit photosynthesis was published by Blanke and Lenz.[53] They found that external fruit chloroplasts are similar to those of sun leaves but scarce and with few grana while chloroplasts placed in the interior of the fruit are adapted to shade.[53] Chlorophyll fluorescence in fruits has been largely used as an important tool for their quality assessment during harvesting and post-harvesting periods. In particular, variable chlorophyll fluorescence and the corresponding photosynthetical parameters derived from Kautsky kinetics have been extensively analyzed.[54,55,56]

Chlorophyll fluorescence in apples was thoroughly studied. For Starking Delicious apples, it was found that F_0, F_m and F_v/F_m decreased with time during the harvest time. A correlation between post storage F_v/F_m and firmness was also observed.[57] DeEll *et al.* showed that methods based on chlorophyll fluorescence could detect stress in apples caused by low or high oxygen concentration and by high carbon dioxide content.[58,59]

For mangoes, a decrease in F_0 and F_m parallel to an increase in internal CO_2 content was found as a function of time, before harvest.[60]

Nedbal *et al.* demonstrated that it was possible to predict lemons quality by chlorophyll fluorescence imaging.[61]

The spectral shape of the original (non-variable) chlorophyll fluorescence, F_0, has been carefully studied for some fruits as apples and kiwis. A physical model to correct the fluorescence spectrum of Granny Smith apples for light re-absorption processes has been developed by Ramos and Lagorio.[62] A very interesting point comes out from this work when analyzing the fluorescence ratio $F_{red}/F_{far\text{-}red}$ compared to those

of green leaves. Exciting in the blue (470 nm) an average value of 1.25 was obtained for apples in comparison with a value of 0.77 for sun-leaves of *Ficus benjamina*. This result is consistent with higher chlorophyll content in leaves leading to higher re-absorption of the emission band at 680 nm. Upon correction for light re-absorption, the fluorescence ratio became similar to a value of 2 for both systems. These results nicely agree with the fact stated by Blanke and Lenz regarding the similarity of external fruit chloroplasts with those of sun leaves.[53]

The chlorophyll fluorescence of Kiwi fruits has been also thoroughly studied and modelled. Emission originated both in the pulp and in the peel was reported, but while variable fluorescence was recorded in the former, no induction of Kautsky kinetics was found for the peel. Values for the fluorescence ratio (red/far-red) corrected for light re-absorption for the pulp were higher than those obtained for the peel and similar to shaded leaves, probably due to the light filtering effect of the peel during ripening.[35] Some authors attributed this high ratio to a reduction in the size of PSI antennae chlorophyll.[56]

Figure 5

Figure 5. Kiwi fruit displays chlorophyll fluorescence. Photograph reproduced with permission of the copyright owner: Chris Williams.

Chlorophyll fluorescence has also been detected and studied in *Pyrus communis* L. (pears),[63] *Musa* L. (bananas),[63] *Persea americana* Mill. (avocado),[63,64,65] *Cucumis melo* L. (cantaloupe),[65] *Fragaria* × *ananassa* (strawberries),[66] *Citrus reticulata* (tangerine),[65] tomatoes[65,67] and cucumbers.[68]

In *Capsicum annuum* L. (green bell pepper) high blue fluorescence (maximum at 440 nm), low green fluorescence and low chlorophyll fluorescence was detected. The experimental fluorescence ratios $F_{red}/F_{far\text{-}red}$ (without correction for light re-absorption processes) are in the order of 0.5 to 0.7 similar to green plant leaves. The blue green emission in this case is probably due to the presence of hydroxycinnamic acids, especially ferulic acid like in leaves.[69]

A nice review of the practical applications of chlorophyll fluorescence in fruits may be found in reference 56.

Fluorescence from other pigments different from chlorophylls has also been found for fruits. Lipofuscin, emitting at 440-470 nm upon excitation at 360-380 nm (Table 1), has been reported to accumulate during the ripening of banana and pear in peels and pulps.[70] Lipofuscin is a final product of autoxidation of cells components consisting in lipids and biomolecules containing residues of lysosomal digestion.[70-72]

Flowers

Four major groups of pigments are present in flower petals: betalains, carotenoids, flavonoids and chlorophylls.[73]

Fluorescence has been reported for petals containing betalains and flavonoids (Table 1). Thorp *et al.* reported also fluorescence from the flowers nectar and they suggested that this emission could act as a visual attractive signal for bees.[74]

Betalains are water soluble nitrogenous pigments present in flowers and fruits of plants of the order *Caryophyllales*, where they replace the anthocyanins (they are mutually exclusive). Within this group, the yellow betaxanthins are formed by a betalamic acid unit attached to different amino acids or amines while red violet betacyanins have a closed structure cycle-dihydroxyphenylalanin (see Table 1).[75]

The physicochemical properties of these compounds have been extensively studied in literature as food additives, mainly in terms of their stability[76] but also about the functionality of these dyes in plants and human nutrition.[77] Regarding their optical properties they have been reported to be responsible for fluorescence emission in some flowers. In this sense, Gandía Herrero *et al.* have been making an important contribution not only in the study of intact flowers,[78,79] but also in the development of methods for extraction and quantification of these dyes.[80,81,82]

The excitation spectrum for betaxanthins in aqueous solution displayed a maximum at 470 nm and their emission band was found around 510 nm. The first report of emission from them was on flower petals of *Lampranthus productus* and *Portulaca grandiflora* (observed *in situ* using confocal fluorescence microscopy).[78] In another work, these authors found that the fluorescence (caused by betaxanthins) and its partial re-absorption in *Mirabilis jalapa* petals formed a pattern that could have implication in the behaviour of pollinators visiting these flowers.[79]

Ono *et al.* have reported fluorescence emission from aurones (a kind of flavonoid) in *Antirrhinum Majus.*[83]

Aurones are a group of natural bright yellow pigments and appear in a few families of plants, particularly in *Scrophulariaceae, Plumbaginaceae* and *Compositae*. Shanker *et al.* studied the spectroscopy of aurones in solution and they found fluorescence quantum yields in the order of 0.011 and 0.002 for different aminoaurones in ethanol (see Table 1).[84]

Anthocyanins, belonging to the group of flavonoids are responsible for most of the reddish colour present in leaves, fruits, flowers and grains.[85] They have been extensively studied since their presence is closely linked to the processes of senescence of leaves, plant stress and antioxidant capacity[86] and their emissive properties in plant

extracts have been presented by Drabent *et al.* in 1999.[87] Iriel and Lagorio studied the optical properties of *Rhododendron indicum* petals (see Table 1).[88] These last authors found absorption at 533 nm due to anthocyanins and a strong UV absorption due to the presence of phenolic compounds other than anthocyanins. Additionally, upon UV excitation, two emission bands were found: at 624 nm (due to anthocyanins) and around 400-500 nm (from other flavonoids). The fluorescence quantum yield for the blue emission was 7.6×10^{-5} to 6.0×10^{-4} in intact white petals, while the fluorescence quantum yield for the red emission varied from 2.4×10^{-5} to 1.9×10^{-4} in pink-coloured petals, depending on their anthocyanin concentration (for the lower content, the higher quantum yield).[89]

In an extension of this work, several intact flowers containing the pigments mentioned above were carefully studied in terms of their fluorescence and reflectance properties: *Bellis perennis* (white, yellow, pink, and purple), *Ornithogalum thyrsoides* (petals and ovaries), *Limonium sinuatum* (white and yellow), *Lampranthus productus* (yellow), *Petunia nyctaginiflora* (white), *Bougainvillea spectabilis* (white and yellow), *Antirrhinum majus* (white and yellow), *Eustoma grandiflorum* (white and blue), *Citrus aurantium* (petals and stigma), and *Portulaca grandiflora* (yellow). For all these cases fluorescence was negligible compared to reflectance and it was again concluded that this evidence plays against a role of biosignaling for the fluorescence in flowers. The highest fluorescence quantum yields were obtained for the ovaries of *O. thyrsoides* (Φf=0.030) and for *Citrus aurantium* petals (Φf=0.014) and stigma (Φf=0.013).[90]

After a quantitative estimation of emerging photons from the petals, fluorescence resulted negligible compared to reflected photons and for this, its role as biosignal towards pollinators is unlikely.[90]

Figure 6

Figure 6. *Ornithogalum thyrsoides* (upper image) and *Citrus aurantium* (lower image), under ambient light (left) and UV light (right)

Regarding carotenoids, lipophilic secondary metabolites that accumulate in plants organs giving red, orange and yellow colours, they have been considered non-fluorescent compounds for a long time. Wolf and Stevens reported that β-carotene, lutein, lutein epoxide and violaxanthin emitted fluorescence in the range 300 to 400 nm upon excitation at 280 nm.[91]

Gillbro and Cogdell reported emission from β-carotene, rhodopin and spheroidenone in carbon disulfide at 580 nm when excited at 520 nm (see Table 1). However, it should be noticed that measured fluorescence quantum yields were extremely low, in the order of 3 to 6 x 10^{-5}.[92] The low fluorescence quantum yield and the overlapping between carotenoids emission and the absorption of other pigments present in plants, probably combine themselves to avoid observation of carotenoids emission in intact flowers.

Animals

Avian

Fluorescence has been detected in some birds feathers. Völker was one of the first describing the fluorescence of parrots plumage by 1937.[93] This fluorescence has been attributed to the presence of psittacofulvins, lipid-soluble pigments which are presumed to be synthesized in the follicular tissue of growing feathers. Psittacofulvins bear some structural similarity with carotenoids but the former show only one absorption

maximum around 420-450 nm while carotenoids display three absorption maxima in their UV-visible spectra (in the range from 400 to 480 nm). Additionally, psittacofulvins, differing from carotenoids, are not obtained from the bird´s diet, but are synthesized in its organism.[94,95]

Pearn *et al.* [96] analyzed the role of the ultraviolet-A induced fluorescence in the appearance of wild-type budgerigar plumage. They found strong UV-A-excited fluorescence from the yellow crown, with an emission peak in the green at 527 nm, and from the white chest feathers (hidden beneath the green external plumage) with emission peak in the blue at 436 nm. To explore the contribution of fluorescence in the optical signaling process, they compared the reflectance from the yellow crown, in the visible part of the spectrum, using illuminants with and without UVA finding no differences between them. These results suggested that UVA-induced fluorescence did not play any signaling role in that case. On the other hand, they did not find emission neither from the green chest nor from the blue tail.

The spectroscopy based results from Pearn *et al.*[96] contradict the previous observations from Arnold *et al.*[97] who performing a behaviour-based experiment with budgeriars, found sexual preference for fluorescent birds, both for males and females. They worked with two groups of avian: one treated with a non UV absorbing petroleum jelly (retaining fluorescence) and another group with reduced emission by application of sunblock which decreased the absorption of UV light. They assured that the groups differed only in terms of fluorescence, but not in UV reflectance. However, some doubts may arise regarding the possibility that the sunblock might have decreased also UV reflectance from plumage. In a later work,[98] Pearn *et al.* studied the budgerigar mate choice using an approach which separated the removal of UVA reflectance from the removal of fluorescence. They concluded that when UVA reflectance was absent,

females did not use fluorescence as a tool for mate choice. Even though, they suggested that the presence of fluorescent pigments absorbing in the UVA might have a functional effect, increasing colour contrast (for a UV sensitive species), when placed near a UV reflecting tissue as calculated by Hausmann *et al.*[98]

The role of UVA reflectance in sexual attraction has been proved to be definitely relevant in zebra finches *Taeniopygia guttata,*[100] starlings *Sturnus vulgaris,*[101] blue throats *Luscinia svecica,*[102] blue tits *Parus caerulus,*[103,104] pied flycatchers *Ficedula hypoleuca*[105] and the budgerigar *Melopsittacus undulatus*[106] but the role of fluorescence in avian remains still now ambiguous.

In 2012, Barreira *et al.* reported fluorescent and ultraviolet sexual dichromatism in a blue-winged parrotlet *Forpus xanthopterygius* distributed in the central and eastern parts of South America. In this work, the absolute fluorescence quantum yields were measured for different patches of plumage both for males and females exciting in the UVA (350-360 nm). The blue rump of the male (reflectance maximum at 465 nm) emitted indigo fluorescence (fluorescence maximum around 430 nm) with a quantum yield of 0.042. The green chest of the male (reflectance maximum at 558 nm) emitted green fluorescence (maximum around 525 nm) with a quantum yield of 0.035. Displaying much lower fluorescence, the chest and rump of the females emitted green fluorescence with quantum yields of 0.012 and 0.010 respectively.[106]

Usually, plumage appearance is more colourful in males than in females. The highest fluorescence found for males of *Forpus xanthopterygius* would also reinforce the observation that nature attempts to highlight bird males compared to females with a more prominent appearance. This fact has been interpreted as the evolution through sexual selection.[108] Other authors considered that cryptic females avoid excessive exposition to predation during the nesting period.[109]

The work of Barreira *et al.* is the first reporting fluorescent sexual dichromatism in avians. The fluorescence of the blue plumage patch is particularly interesting because it might be structural and not due to pigments. This point is still unresolved and opens an exciting area of study.

Mc Graw *et al.* have reported fluorescence emission from yellow penguin feathers and it was attributed to the presence of spheniscin (see Table 1).[110,111] The three-dimensional structure of the most abundant spheniscin was determined by two-dimensional NMR and molecular modelling techniques have been reported by Landon *et al.*[112]

Butterflies and moths

The first studies reporting fluorescence in insects were made in the 20s by Mottram and Cockayne.[113] Following, Cockayne analyzed the butterfly collection of the Museum of England under UV light observing their fluorescence.[114] Subsequently, Phillips reported the colour of the observed emission for a collection of 3122 specimens from 10069 different species of moths and butterflies, belonging to the order *Lepidoptera*.[115]

Bright fluorescence is seen in the yellow parts of the wings of *Papilio xuthus*, *P. helenus*, *P. protenor* and others (family *Papilionidae*), and in the wings of *Euripus*, *Parhestina*, *Myner* (family *Nymphalidae*) among others. In the case of *Pieridae* family, the pigments responsible for the wing pigmentation are pterins (a class of substituted pteridines).[116] Pterins (Table 1) are highly insoluble under physiological conditions and therefore they are commonly deposited as granules.[117]

The pteridines are metabolic products of purines and are present in many insects. They absorb light around 360 nm and fluoresce in the blue.[118]

A remarkable fluorescence is particularly observed for Swalowtails (*Papilio*) butterflies, which belong to the *Princeps nireus* species. They live in eastern and central Africa and have black wings with bright blue patches. Under illumination at 420 nm they display a strong blue-green emission (maximum at 505 nm) which was studied by Peter Vukusik and Ian Hooper at the Exeter University. The high intensity of this fluorescence is due to a marvelous structure of their wing scales. In fact, in the coloured patches of the wings, there are scales that function as a 2D photonic crystal consisting of a slab of hollow air cylinders (mean diameter 240 nm and spacing 340 nm) where the fluorescing pigment is infused. The slab produces a high directionality of the fluorescence outwards. Additionally, the scales have a sort of mirror surface (three layers of cuticle) underneath, acting as a Bragg reflector and reflecting the fluorescence travelling downwards.[119]

Curiously, the way that nature uses to direct and enhance the intensity of fluorescence in these butterflies is analogous to that used in commercial LEDs.[120]

A similar behaviour was reported for the fluorescence in the butterfly *Morpho Sulkowskyi*, which lives in South America and has white wings with blue patches[121] and in the wings of the male *Troïdes magellanus.*[122]

Beetles

Lawrence observed in 1954 that some species of beetles and dragonflies had white and yellow spots that emitted blue fluorescence when exposed to UV radiation.[123]

Fluorescence in beetles is induced with light wavelengths from 360 nm to 480 nm and emitted usually in the blue-green or green-yellow (460 to 625 nm).[124,125]

Fluorescence emission was also detected in the juvenile stages of flies and beetles (eggs and larvae) and it is suspected to be related to the fact that cuticle is not

yet sclerotized in young specimens. Presently, this property is used as an early detection tool for the presence of flies and beetles in food by a nondestructive method[126]. The optical properties of the compound responsible for the emission, presented similarities to pterins (widespread in insects) displaying an absorption maximum at 345-350 nm and an emission band centered at 421-427 nm.

Recently, Welch *et al.* published a complete review on insect fluorescence where its evolution and functionality are discussed. This important work contains emission spectra recorded on intact insects.[127]. These authors explained that in general, emission spectra for the isolated fluorescing compounds in solution are distorted with respect to those observed for intact samples and they are usually pH dependent.[128]

Furthermore, the characteristics of the insect surface on which the fluorophore is placed are critical since they can significantly affect the intensity and distribution of the emitted radiation and thereby the visual signal in the observer. In this sense, some researchers published the visual effect from fluorophores confined in three-dimensional photonic structures as those found in the beetle species *Celosterna pollinosa sulfurea* and *Phosphorus virescens*.[125]

Dragonflies

In addition to the pterins listed above, dragonflies usually contain another fluorescent compound of protein structure (also spread in other invertebrates) having an emission in the blue and related to the flexibility they possess in their wings.[129] This compound is of great interest in the technology area for its excellent properties as it plays an important role in jumping, flying and generating sounds of insects.[130] It presents different visual patterns that may in turn facilitate intraspecies recognition.

Millipedes

Millipedes contain a fluorescent compound derived from the pterins in their cuticles. In some species, as in *Luminodesmus sequoia*, the fluorescence process takes place from a chemiluminescent reaction which provides the light energy that is absorbed by the fluorescent 7,8-dihydropterin-6-carboxilic acid. This last compound is located in the cuticle and it is unstable out of it (leading to pterin-6-carboxylic acid). In other cases, as in *Parafontaria laminata armiguera*, chemiluminescence does not take place and fluorescence is the result of direct absorption of UV light. In this case, a blue emission at 455 nm is produced upon excitation. The compound pterin-6-carboxylic acid has been found in their cuticle.[131]

Regarding the potential role of this fluorescence, the case is somewhat disconcerting since these specimens are blind and emission has no value as a visual signal. Additionally, they eat leaves and they are not expected to attract any insects for food.

Cockroaches

In the brain of crustaceans and cockroaches a fluorescent compound called lipofuscin has been found.[132] Willis and Roth have also reported fluorescence from cockroach guts.[133] Due to the internal character of the components responsible for this fluorescence, it seems that this emission, in both described cases, would serve no role.

Bees

Fluorescing pigments have been found in the thoraces of *Apis Mellifera* bees. Higher concentrations were found for hive and forager bees and lower for queen and drones (which have lower flight activity). They are Schiff-bases with excitation maximum

around 370 nm and emission at 445 nm. These pigments are products of the oxidation of polyinsaturated lipids and their concentration is higher for older bees.[134]

Nemesio studied orchid bees, 13 species of *Eulaema* and 12 species of *Eufriesea,* but only *Eulaema niveofasciata* specimens presented fluorescence.[135] In this work, Nemesio speculated that bee fluorescence might have a role as biosignal in mating and as warning to predators. Nemesio also suggested that the low probability of finding fluorescent bees (1 out of 25 of the studied cases) in forest areas is due to the lack of UV in these environments. He also pointed out that fluorescence might be found more frequently in bee species typical of open, well illuminated environments.

Spiders and scorpions

The emission from the cuticle of the scorpions was known by geologists and people related to mining activities from ancient times. Lawrence began to address this issue in a more systematic way in the 50´s by studying the scorpions preserved in the National Museum of Bulawayo in Rhodesia. He found that they emitted a pale green colour when illuminated with UV radiation.[136]

Blue fluorescence was also observed in spider patches. Differing from spiders, scorpion fluorescence came from their cuticle while the intersegmental membranes remained dark upon irradiation with UV light.[136] This cuticle emission was attributed to the presence of a beta carboline (tryptophan derivative)[137] and a coumarine derivative (see Table 1).[138]

Scorpion eyes have their higher sensitivity in the green at wavelengths similar to their fluorescence. Gaffin *et al.* proposed that scorpion fluorescence was related with their perception of light, but further studies should be carried out in this area to reach a complete understanding of its biological function.[139]

Fluorescence from spiders was reported by Andrews *et al.* when exciting with UV light.[140] Fluorophores were present in spider´s haemolymph but strong emission took place when the fluorophores were sequestered in their setae or cuticle. Excitation of fluorophores from different spider species was achieved with wavelengths from 288 to 333 nm and the emission peaks varied from 325 to 466 nm according to the species. They proposed that the evolution in spiders may have been driven from natural selection imposed by predator-prey interaction. Lim and Land studied the courtship behaviour of the spider *Cosmophasis umbratica* in the presence and absence of UV light showing very interesting results. Males and females of this species differ in their spectroscopic properties. Interestingly, males have UV reflective patches that are absent in females while females present a UV-excited green fluorescent in their palps, which is missing in males. By means of filters, Lim and Land prepared a set of experiments removing selectively UV reflectance from males or UV-excited fluorescence from females. They found that the courting pair response decreased appreciably when either the UV reflection from males or when the green fluorescence from females were selectively removed. These results provided evidence in supporting a role of sexual attraction for fluorescence in spiders.[141]

Sea organisms

To date, fluorescing marine organisms have been reported in four phyla. The first fluorescent protein, the green fluorescent protein (GFP) has been discovered in the class *Hydrozoan*, in the bioluminescent hydromedusa *Aequorea victoria,* belonging to the phylum Cnidaria (see Table 1).[142] In this case, the processes of bioluminescence and fluorescence are both present and strongly connected. In fact, the blue light originated inside the bioluminescent cells by the photo protein aequorin (calcium-activated

luminescent complex) is absorbed by the green fluorescent protein (GFP) which finally emits green light.[143]

Over time GFP analogs have been found in other classes of *Hydrozoans* and in many organisms belonging to the class *Anthozoa* (for a complete description see references 144 and 145).

Examples of colonial *Hydrozoans* are *Siponophores*. In 2005, Haddock *et al.* studied the bioluminescence and the red fluorescence of lures in a *Siponophor* that lives deep in the ocean.[146] In this work, three individuals of the gender *Erenna* were studied. The authors could determine that the photophores within the tentillas contained only bioluminescent tissue but when they matured they were surrounded by a red fluorescing substance. The emission spectrum for a mature photophore *in vivo* presented an emission maximum at 620 nm when excited at 410 nm, while the purified extract had a maximum at 583 nm for the same excitation conditions.[147] According to the absorption and emission spectra of the fluorescing compound in *Erenna*, the authors suggested that its chemical structure should be similar to a porphyrin as found in jellyfish and fish.[148] With respect to the biological function of this red fluorescence since the filaments exhibit a characteristic flicker, it was concluded that this *Siponophor* uses these flares as lures to attract fishes.[146]

In 2004, Shagin *et al.* reported the development of six GFP homologs in organisms of the phylum Arthropoda: copepods belonging to the family *Pontellidae*. In these species the biological function of fluorescence would be the recognition among individuals of the same species.[149]

Recently Mazel *et al.* published a study on the Mantis Shrimp (*Lysiosquillina glabriuscula*), a stomatopod crustacean which lives in the western Atlantic from South Carolina to Brazil. Mazel *et al.* has reported fluorescence from Mantis patches.[150] This

fluorescence presented an excitation spectrum with maximum at 440 nm and emission peaking at 524 nm, a wavelength well transmitted by sea water. These stomatopods have several photoreceptors (a number of at least 8 are reported in that work). Taking into account their sensitivity, the authors reported that at a depth of 40 m the fluorescence signal contributed in 12% of the photons that were absorbed by the photoreceptor with maximum at about 530 nm and 30% of the photons absorbed by the photoreceptor with maximum at 590 nm, concluding that fluorescence did increase signal brightness at great depths.

In 2007, Deheyn *et al.* found fluorescing proteins in a third phylium: Chordata. They reported the existence of an endogenous green fluorescent protein (GFP) in three cephalochordate amphioxus species collected in three geographically widely separated sites: *Branchiostoma floridae* collected in Tampa (Florida), *Branchiostoma lanceolatum* collected in Banyuls-sur-Mer (France), and *Branchiostoma belcheri* collected in Enshu-nada Sea (Japan).[151] These authors suspected the presence of endogenous GFPs since they had previously noticed a uniform green fluorescence emission when the eggs and embryos of amphioxus were illuminated with UV light.[152] The fluorescence emission spectra obtained from adults of the three studied species of amphioxus showed emission maxima at 524 nm for *B. floridae*, 526 nm for *B. lanceolatum* and 527 nm for *B. belcheri* when excited with UV light of 380 nm. With regard to the possible functions of these fluorescent molecules the authors suggested two possible alternatives: photoreception and photoprotection against UV radiation and blue light.

More recently, Haddock *et al.* found a photoactivable GFP in the phylium Ctenophora: the bioluminescent comb jellies (*Haeckelia beehleri*). Fluorescing granules in the outer epithelium of the studied organisms could be observed by fluorescence microscopy. The emission spectrum for the protein showed a maximum at 512 nm and a

shoulder at 542 nm, while the absorption maximum was found at 495 nm. The authors speculated a photoprotective role for this fluorescent protein.[153]

Fluorescence is particularly interesting in corals (marine invertebrates). Most of them have fluorescent compounds displaying emission under UVA excitation. This feature can be observed for corals belonging to the order of *Scleractinia* as for other classes of *Anthozoa.*[154] At present, it is thought that GFP-like proteins are responsible for the wide variety of colours that may be observed for *Hermatypic* reef corals.[155,156,157,158] Shagin *et al.* described proteins similar to the GFP in *Cnidaria* and *Bilateria*.[149] According to these authors, homologs of GFP are very similar at the protein structure level coming probably from a common ancestor.

The biological function of fluorescence in corals is actually amazing. Kawaguti suggested a possible role for the enhancement of the available light in the photosynthetical process of the algal symbiont. In fact, the pigments present in the host can absorb short-wavelength light and emit fluorescence at longer wavelengths which can be used in turn by the symbiotic dinoflagellates which live in limiting light conditions.[159]

Salih *et al.* could determine the location of the fluorescent granules in corals by means of fluorescence imaging techniques. For corals acclimated to high light intensities (surface water), these granules were located in the epidermis and the outer part of the endoderm, in a privileged position to filter excess sunlight. On the other hand, in the case of corals acclimated to shade conditions (from deep water), the fluorescent granules were found among algae or below them in the endoderm allowing an increase in the availability of light for algae endosymbionts.[160]

Regarding the filtering effect, Salih *et al.*[160] demonstrated that the fluorescent pigments can exert a filtering function for excessive sunlight by supplementing the

filtering of UVB light caused by mycosporin-like amino acids, (MAAs).[161] MAAs are effective to block wavelengths shorter than 360 nm but they provide limited protection against radiation of longer wavelengths (UVA and blue).[160] Both UVA and the blue region of electromagnetic spectrum can penetrate deep into the sea (20 m or more for UVA and around 100 m for blue light) which can potentially affect the photosynthetic process of symbiotic dinoflagellate algae, producing photoinhibition and generation of reactive oxygen species and even bleaching of corals.[162,163,164,165] Salih *et al.* presented further evidence confirming that excessive sunlight can be dissipated by the fluorescent pigments in corals not only at wavelengths of low photosynthetic activity but also by light reflection and scattering in the visible and infra-red.[154]

Other authors as Mazel and Fuchs explored the influence of fluorescence in the visual perception of coral colours by humans.[156]

Matz *et al.*, on the other hand, studied and modelled the influence of fluorescence in the colour of reef-building corals according to the visual system of fishes which occupied different ecological niches within the reef.[157] Although, it was not the first time that authors speculated on the need of coral reefs to be seen by other species.[166] The relevance of the work of Matz and collaborators lies in the spectrometry measurements made *in situ* and modeling of visual systems in three fish species representative of three forms of life on the reef damselfish (*Chromis ovalis*), butterflyfish (*Forcipiger flavissimus*) and barracuda (*Sphyrena helleri*). These authors concluded that the effect of the fluorescence due to GFP-like proteins on coral colour might be a relevant factor in the visual ecology of the reef fishes.[157]

Beyond all the works published regarding coral fluorescence, its biological function has to be further explored. Frequent controversies are found in bibliography at the moment. While several authors hypothesize a photoprotective effect for the

endosymbiont algae,[154,160], others claim that in some cases the own mechanism of photoprotection of dinoflagellates (accessory pigments of algae could dissipate excess radiation as heat) would exceed the effects of fluorescing proteins.[167,168]

The diversity of colours within the green fluorescent protein-like family was discovered in a kind of non-bioluminescent *Anthozoa.*[169] According to Henderson and Remington, cyan and green fluorescent proteins share the same chromophore structure.[170]

Cyan proteins have an emission maximum between 485 and 495 nm.[144] These proteins display excitation and emission curves wider than those for green proteins and. they have the lower values for the extinction coefficient among the colour proteins. Green proteins have narrow emission bands with maxima at wavelengths around 510 nm and very high fluorescence quantum yields (0.79).[145] Regarding yellow proteins, two wild types with emission maxima between 525 and 570 nm are known.[145] Red proteins also show two structures: DsRed-type or Kaede-type[145] and purple-blue proteins have high extinction coefficients and no fluorescence. Detailed information on the spectroscopy and structure of these proteins can be read in references 144 and 145. Finally, it is interesting to note the work of Field *et al*. who suggested the existence of an evolutionary adaptation between the coral and the algae symbiont which produced as a result the great diversity of colours in the reef-building corals.[171]

Fluorescence has also been found in fishes**.** Red fluorescence in reef fishes has been detected and proposed as a signaling mechanism by Michiels and colleagues.[172] In their work they reported at least 32 fish species displaying red fluorescence in depths where the red component of sunlight was absent because it was absorbed by sea water.

Sparks *et al.* also reported biofluorescence in cartilaginous fishes such as sharks and rays (*Chondrichthyes*) and in bony fishes such as eels and flatfish. They could

identify more than 180 species of fluorescent fish, a fact that highlights the richness of fluorescing species that may be found in the sea.[173] Based on studies on the ichthyofauna from the Caribbean and Pacific Ocean, on living collections in aquariums and on previously published works, these authors concluded that biofluorescence was phylogenetically widespread but it was also phenotypically variable and very common in young crytobenthic coral reefs. According to this research, marine fishes might have red (reported for fishes inhabiting surface water reefs), green or orange fluorescence and even a combination of these patterns, with specific design for each species.[173]

Michiels *et al.*[172] found highly variable fluorescent patterns among reef fishes. They mainly observed fluorescence in the rim of the eyes, parts of the head or chest and in the fins. Dissection of individuals of different species (pipefish, triplefins, blennies, and gobies) showed that the red fluorescence was associated with guanine crystals. With regard to the biological functionality of this fluorescence, the authors argued that the emission of light of a colour that is absent in an environment would allow a fish to increase its contrast against the background.Therefore they proposed red fluorescence as a possible communication or attraction biosignal in the blue environment. The same hypothesis was proposed earlier by Douglas *et al.*, in dragon fish (*Malacoteus niger*), which inhabits deep ocean waters. This particular fish emits blue bioluminescence which is absorbed by the suborbital photophores and then is re-emitted as fluorescence in the far-red.[174] Strikingly, in this case, excitation and emission spectra suggested the presence of a magnesium-free derivative of chlorophyll (pheophorbide-a or pyropheophorbide-a) which would not be biosynthesized but would have a dietary origin.[175]

Sparks *et al.*[173] also pointed to the phenotypic colour variations mentioned above with a possible role in communications or even in mating behaviour.

Other evidence suggesting a function of fluorescence in the intra-specific communication is the fact that many fluorescent fishes as sharks, lizardfishes, scorpionfishes, labrids (wrasses), and flatfishes have intraocular yellow filters which act as long-wave-pass filters, allowing fishes to increase visual contrast and thus seeing the fluorescence patterns of other organisms of the same species, while remaining hidden from the rest of the fishes and possible predators.[176]

Michiels *et al.*[172] also questioned whether the fishes that emit red fluorescence were able to see it. By using microspectrometry in *Eviota pellucida* they could confirm that these specimens showed visual sensitivity to long wavelengths, and that they were able to see their own fluorescence. The occurrence of red fluorescence in fish was found to be greater at higher depths supporting a visual function and not UV protection.[177]

Gerlach *et al.*[178] recently discovered that male fairy wrasses (*Cirrhilabrus solorensis*) responded aggressively to the stimulus of seeing its reflection in a mirror. A less antagonic response was obtained when the fluorescence signal was masked. This experiment clearly showed that fluorescence signal affects the interaction between males of the same species.

Other possible functions for biofluorescence in fishes are proposed by Sparks *et al.*[173]. It is well known that some marine fish spawn synchronously in the light of the moon.[179,180] Moonlight illumination in surface ocean waters could excite green and red fluorescence in fishes and their specific fluorescent pattern might provide a sort of recognition during the spawning stage by fishes of the same species. Showing very attractive pictures and videos, Sparks *et al.* have demonstrated that several sea organisms can use fluorescence as a tool for camouflage. In fact, they have recorded a red fluorescent scorpionfish on a red fluorescing algae and a green fluorescing *Scolopsis bilineata*, close to a green fluorescing *Acropora* coralhead.[173]

Discussion and prospects for future research

As we have discussed above, fluorescence emission is found in different living organisms with diverse features and intensities. We have presented only a limited number of examples among which we considered the most relevant cases. Nevertheless, there are many other fluorescent natural tissues not listed in this work.

Fluorescence in plant leaves cannot be considered as having a role in biosignaling. Instead, the emission from excited chlorophyll-a molecules is one of their deactivation pathways (competing both with heat dissipation and with electron transfer leading to photosynthesis) that is used as a tool to infer plant health.

As a matter of fact, chlorophyll fluorescence in plants deserves a particular attention because under conditions of actinic light illumination, it varies along the time in terms of both its intensity and spectral distribution. This feature, which is caused by its competition with photosynthesis, is unique and strongly differentiates it from other fluorophores in nature. On this basis we can say that the study of chlorophyll fluorescence has still a long way ahead until full and detailed understanding of this phenomenon is achieved.

It is suggestive and very appealing to think in biofluorescence as a communication signal among species or among specimens of the same species. The biosignaling function has been demonstrated in many cases (spiders, sea organisms) and refused or at least questioned in others (flowers). In avian, there is evidence in favour of a role of fluorescence as biosignal but there are still some contradictions that must be clarified. A remarkable point is the finding of fluorescence sexual dichromatism in some of them. In other cases as in millipedes, the potential role of fluorescence, if there is any, is not understood.

Beyond the relevance or not of fluorescence as a biosignal, it is used in many technological applications and it was transformed in an excellent tool to obtain information on the organism, in a non-destructive way, even for low intensities signals. This is the case for plant leaves and fruit fluorescence.

Sometimes, fluorescence is not connected to communication signals at all, but with cell aging as happens with lipofuscin. This compound is not only present in bananas, pears and crustacean brains, as stated above, but also in mammalian cells of different tissues not discussed here (liver, kidney, heart, neuronal tissue, dermal tissue, etc.).[181] The fluorescence of this pigment does not seem to have a biological role but it is used as a marker to estimate age and stress in cells.[182]

Many manuscripts on the analysis of fluorescence in nature have been published but still a lot of future work is needed. Quantitative works with experimental determinations of fluorescence quantum yields *in vivo* are particularly in shortage.

Another interesting point is studying structural fluorescence in natural materials. The designation "Structural fluorescence" was used for butterflies by Van Hooijdonk *et al.*,[122] but they referred to a dye-generated fluorescence enhanced by a structural factor (a photonic structure) and not to fluorescence originated by the structure itself. This kind of analysis is very important and is today under discussion for the plumage of certain avians as the blue emitting patches of parrotlets.

Regarding sea organisms, it may be affirmed that their richness in fluorescence emission is superb and they stand out from other organisms, especially terrestrial species. In fact, marine organisms live in a visual domain restricted to the blue part of the electromagnetic spectrum (around 470 nm) which is the result of the water filtering effect on both the incident and reflected light.[183] According to several authors, this spectral light restriction might help marine organisms to enhance visual contrast from

the fluorescence contribution, a fact that in terrestrial environment would be more difficult to achieve.[150,173,184]

It should be noticed that lately, the GFP has been the star among fluorescing compounds. The discovery of GFP-like proteins with different structures, extracted from other organisms may have great relevance in science. In fact, there is much interest in the development and study of new fluorescent proteins due to their applications in biotechnology, in molecular biology and in monitoring cellular processes by microscopy-based techniques.[185,186] Furthermore, it is still necessary to broaden the understanding of their roles in nature learning more about their biological functions while expanding knowledge about the distribution of these proteins in the phylogenetic tree of life.[145,149]

Last but not least, it should be noticed that unveiling the origin, features and function of fluorescence in nature requires a highly interdisciplinary work in which biological, physical and chemical focuses should be combined for a complete understanding of the systems.

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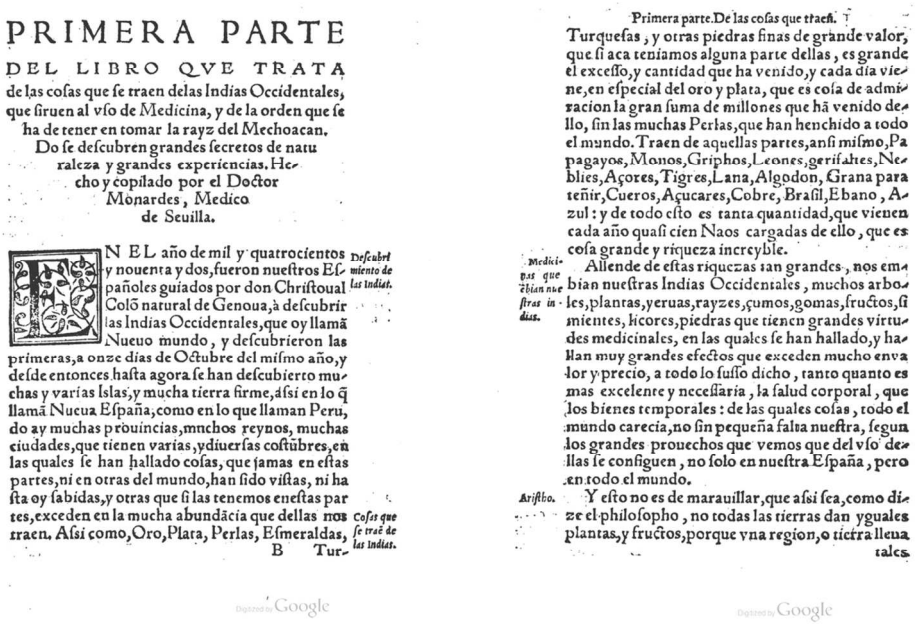

PRIMERA PARTE
DEL LIBRO QVE TRATA
de las cosas que se traen delas Indias Occidentales, que siruen al vso de Medicina, y de la orden que se ha de tener en tomar la rayz del Mechoacan. Do se descubren grandes secretos de naturaleza y grandes experiencias. Hecho y copilado por el Doctor Monardes, Medico de Seuilla.

EN EL año de mil y quatrocientos y nouenta y dos, fueron nuestros Españoles guiados por don Christoual Colõ natural de Genoua, à descubrir las Indias Occidentales, que oy llamã Nueuo mundo, y descubrieron las primeras, a onze dias de Octubre del mismo año, y desde entonces hasta agora se han descubierto muchas y varias Islas, y mucha tierra firme, assi en lo q̃ llamã Nueua España, como en lo que llaman Peru, do ay muchas prouincias, muchos reynos, muchas ciudades, que tienen varias, y diuersas costũbres, en las quales se han hallado cosas, que jamas en estas partes, ni en otras del mundo, han sido vistas, ni hasta oy sabidas, y otras que si las tenemos eneestas partes, exceden en la mucha abundãcia que dellas nos traen. Assi como, Oro, Plata, Perlas, Esmeraldas, Turquesas, y otras piedras finas de grande valor, que si aca teniamos alguna parte dellas, es grande el excesso, y cantidad que ha venido, y cada dia viene, en especial del oro y plata, que es cosa de admiracion la gran suma de millones que hã venido dello, sin las muchas Perlas, que han henchido a todo el mundo. Traen de aquellas partes, ansi mismo, Papagayos, Monos, Griphos, Leones, gerifaltes, Neblies, Açores, Tigres, Lana, Algodon, Grana para teñir, Cueros, Açucares, Cobre, Brasil, Ebano, Azul: y de todo esto es tanta quantidad, que vienen cada año quasi cien Naos cargadas de ello, que es cosa grande y riqueza increyble.

Descubrimiento de las Indias.

Cosas que se traẽ de las Indias.

Medicinas que tienen nuestras Indias.

Allende de estas riquezas tan grandes, nos embian nuestras Indias Occidentales, muchos arboles, plantas, yeruas, rayzes, cumos, gomas, fructos, simientes, licores, piedras que tienen grandes virtudes medicinales, en las quales se han hallado, y hallan muy grandes efectos que exceden mucho enualor y precio, a todo lo susso dicho, tanto quanto es mas excelente y necessaria, la salud corporal, que los bienes temporales: de las quales cosas, todo el mundo carecia, no sin pequeña falta nuestra, segun los grandes prouechos que vemos que del vso dellas se configuen, no solo en nuestra España, pero en todo el mundo.

Aristho.

Y esto no es de marauillar, que assi sea, como dize el philosopho, no todas las tierras dan yguales plantas, y fructos, porque vna region, o tierra llena tales

Figure 1. Introductory fragment from the book written by Nicolás Monardes in 1565: Historia medicinal de las cosas que se traen de nuestras Indias Occidentales que sirven en Medicina, Primera parte.
114x77mm (300 x 300 DPI)

Figure 2. Chlorophyll-a emits red and far red fluorescence in vivo under UV or blue excitation.
112x88mm (300 x 300 DPI)

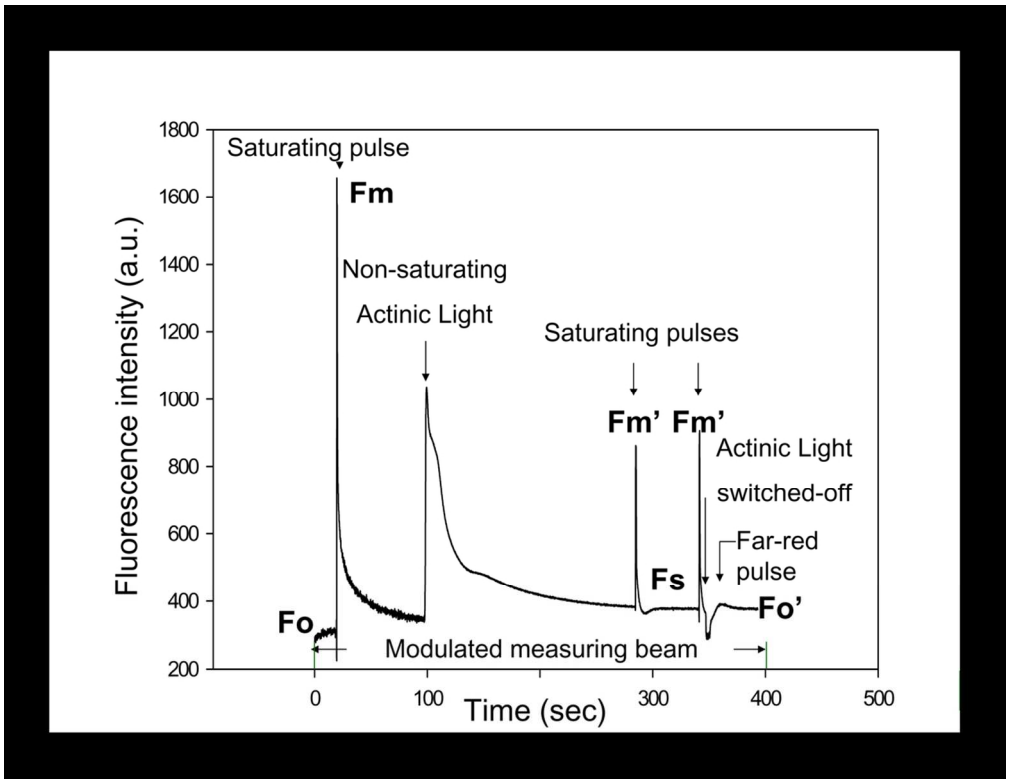

Figure 3. Variable chlorophyll fluorescence recorded with a pulse-modulated fluorometer for a typical plant leaf. Reproduced from reference 18. For a detailed description of this process see references 17-19.
107x83mm (300 x 300 DPI)

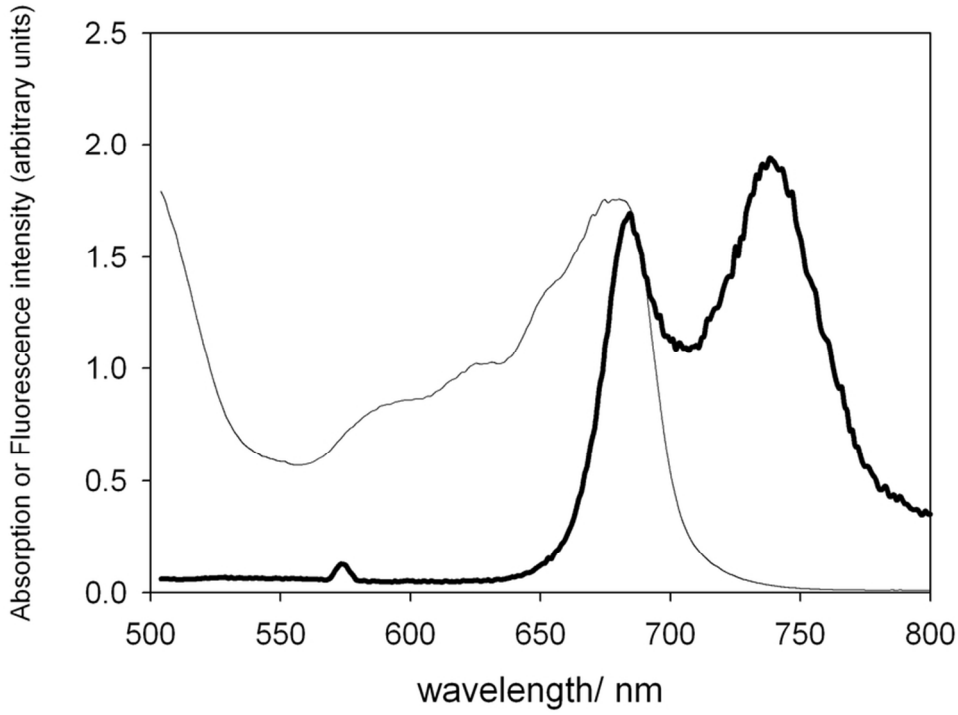

Figure 4. Absorption spectrum (thin line) and F0 fluorescence emission spectrum corrected by the detector response to wavelengths (thick line) for a leaf of Ficus benjamina. Excitation wavelength: 460 nm.
80x64mm (300 x 300 DPI)

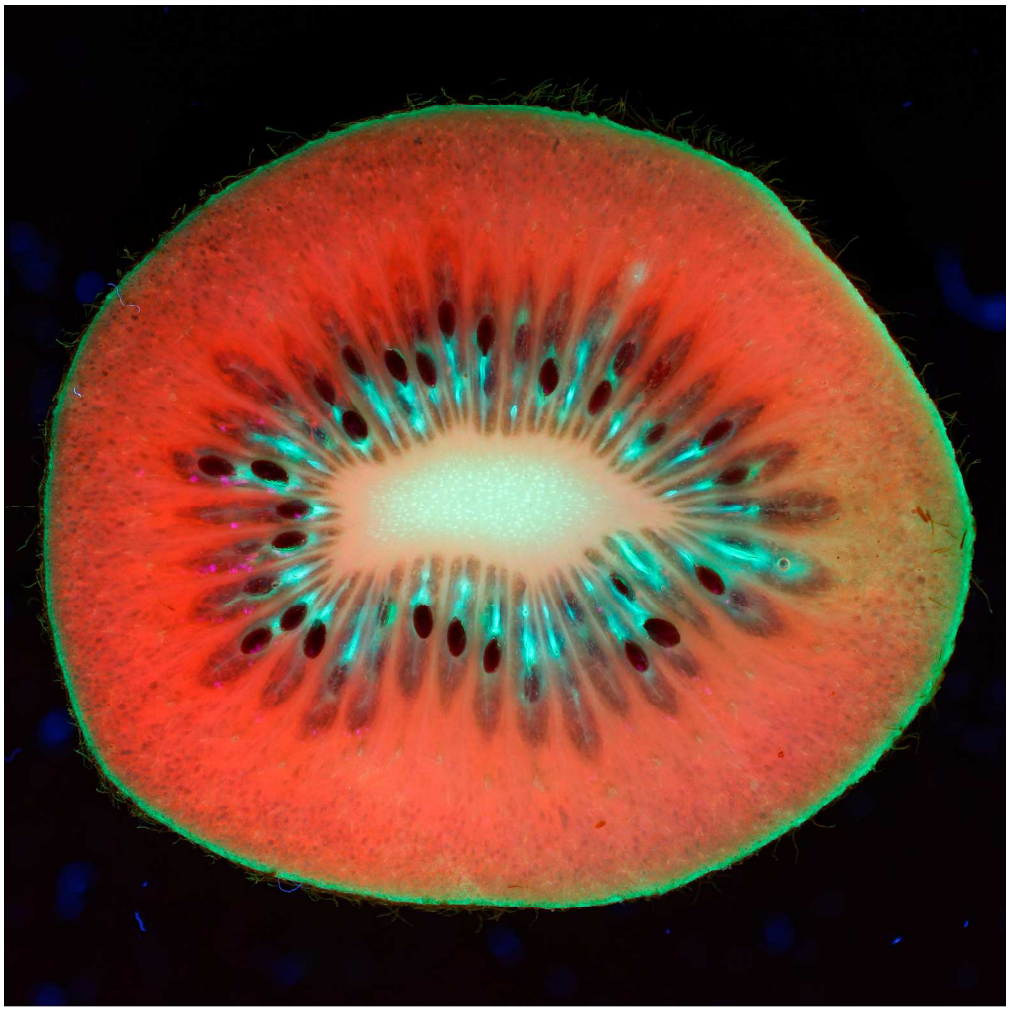

Figure 5. Kiwi fruit displays chlorophyll fluorescence. Photograph reproduced with permission of the copyright owner: Chris Williams.
156x156mm (300 x 300 DPI)

Figure 6. Ornithogalum thyrsoides (upper image) and Citrus aurantium (lower image), under ambient light (left) and UV light (right)
126x88mm (300 x 300 DPI)