Review
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On the blue coloration of vertebrates†

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Summary

Although the various vertebrate classes, from fishes to mammals are each distinctive, they possess many common features making it important to understand their comparative biology. One general feature that has long commanded interest is the integumental pigmentary system. Thus, much is known about particular pigment cells; however, the basis for some specific colors, such as blue, has escaped the scrutiny of the comparative approach. Regardless of Class, blue is almost always a structural color based upon incoherent or coherent scatter of blue wavelengths from the animal surface. The source of scatter may be intracellular or extracellular. A main intracellular scatterer is the surface of reflecting platelets of iridophores of lower vertebrates. Extra-cellular scatter is widespread and thought to occur from ordered dermal collagen arrays in primitive fishes, birds and mammals including humans. Among birds, feather structures provide major means for extra-cellular light scatter. There is only one known example of blue color deriving from a blue pigment found within a pigment cell. For amphibians, reptiles and birds, the scatter of blue wavelengths, together with the presence of yellow pigmentation, is fundamental for the expression of green coloration.

Key Words: blue color/vertebrates/iridophores/collagen/dermis/green color/feathers

Introduction

The bright colors of animals have long fascinated humans of all ages. Adults and children alike revel in the striking reds, yellows and blues of organisms as diverse as butterflies and birds and in almost every home, at one time or another, an aquarium housing brightly colored fishes can be found. Scientists have likewise been drawn to these striking creatures and many studies over the last 150 yr or more have led to the purification and identification of many of the pigments involved (Fox, 1976; Fox and Vevers, 1960). A cataloging of these pigments reveals a profound paucity of blue pigments; nevertheless, blue is a very common color among many animals. In vertebrates, blue coloration is manifested in an array of different species ranging from primitive fishes to man. In the great majority of cases, this blue coloration results from physical phenomena and thus, blue is almost always a structural color-based largely on selective light scatter from surface elements that differ in refractive index. Blue is manifested as a normal color of many species or as a mutation in others. In man, blue coloration occurs in specific dermatological conditions often referred to as cerulodermas. These are often congenital anomalies that include a variety of blue nevi such as the Nevus of Ota and Mongolian Spots. The blue colors of such cerulodermas are generally considered to be of structural origin (Prum and Torres, 2004). The blue colors of lower vertebrate taxa, especially fishes, are almost always structural colors (Fujii, 1993a,b). A singular exception occurs in two species of callionymid fish, the mandarin fish, *Synchiropus splendidis*, and the psychedelic fish, *S. picturatus* (Goda and Fujii, 1995). Here, a blue pigment is found in novel chromatophores called, cyanophores. Overt blue coloration in amphibians and reptiles is

†The essence of this review is derived from a mini-symposium, 'The Blues Symposium', presented at the XVIth International Pigment Cell Conference held in Anaheim, CA, USA, October 29 to November 3, 1996. It was organized by Joseph T. Bagnara and co-organized by Jean L. Bolognia and the late Yoshiaki Hori (to whom this review is dedicated). Other speakers were Craig Bohren, Ryozo Fujii, Philip J. Fernandez, Randall L. Morrison, and Walter C. Quevedo, Jr.

‡Professor Ryozo Fujii lost his battle with cancer on July 1, 2002, and thus his contribution here is posthumous. Were it not for his enthusiasm toward making the scientific community aware of the bases of blue coloration is fishes, this review may not have been written. We are grateful to his daughter, Sana Harada, for providing materials and preliminary drafts of manuscripts in preparation.

much less common than in fishes; however, the common green color of many of these species is based upon the emanation of blue wavelengths from chromatophores on the dorsal surface (Bagnara et al., 1968). In these cases, the source of blue color derives from specific chromatophores, notably, iridophores which when covered over by yellow pigment cells, xanthophores, results in green coloration (Bagnara and Hadley, 1973). An amusing confirmation of this fact is described by Fox and Vevers (1960) who record that an Australian tree frog was sent in alcohol to a museum to be described and named. Since it was bluish, it was named *Hyla coerulea*. However, in nature this frog (now *Litoria coerulea*) is actually green, but in the museum specimen, the alcohol had dissolved away the yellow screening pigment. Among avian species, blue colors are seen in the plumage of a variety of species, but probably less so than are greens. Blues are also seen in avian skin where they are often quite spectacular. As in amphibians and reptiles, the green colors of birds may depend upon the reflectance of blue wavelengths that pass through a filter of yellow carotenoid pigments in the feathers (Fox and Vevers, 1960). However, recent studies have revealed that green and yellow hues of many avian species are structural colors (Prum and Torres, 2003a,b).

The aim of this review of blue pigmentation is to emphasize how commonly this color is found among a diversity of vertebrate species. It is intended to show that notwithstanding a broad taxonomic separation between groups, the physical and chemical elements upon which the expression of blue colors are based, is often the same or quite similar. It will not be possible to cover all vertebrate classes to the same depth; however, each class will be touched upon to some degree. As much as possible, emphasis will be placed upon the presence of similar mechanisms involved in the manifestation of blue coloration among these disparate groups. One such similarity resides in the fact that the basis of blue coloration in blue nevi in humans seems to be the same as that responsible for blue spots in a primitive fish.

Structural colors

Animals appear blue in color because the wavelengths of light that reflect from their respective surfaces are dominated by blues and violets (*c.* 400–550 nm). In the majority of cases, the expression of these blue wavelengths is a function of the morphology of the surface of these organisms and thus, the emanated colors are called structural colors. In invertebrates, blue coloration is sometimes pigmentary resulting from the presence of true pigments in the integument and appendages (Needham, 1974). There is only one case that we know of among vertebrates where blue color is due to the presence of a true blue pigment. This is the case of callionymid fishes that will be discussed later.

Although structural colors have long been recognized as the principal basis for blue coloration of vertebrates, the scope of this review limits the depth of coverage of the physics involved. Light scatter will be discussed in simple terms sufficient to allow an appreciation of its role in the production of blue coloration, especially in birds and mammals, and in the blues and greens of lower vertebrates. Following the lead of Prum and Torres (2003a,b, 2004), the simplest classification of mechanisms for the production of structural color uses the terms of either incoherent or coherent scattering of light (Bohren, 1987; Bohren and Huffman, 1983). Incoherent light scatter takes place when individual light scattering objects, particles or small surfaces, are randomly separated from one another by an average distance that is large relative to the wavelength. The particle size distribution will determine the spectrum of the light reflected by the medium (or tissue structure). For the best production of blue light, the suspension of particles should overlay something dark. For example, in the skin, blue production is best when the scattering objects are underlain by melanin. Coherent scattering of light occurs when the distribution of light scattering elements is precisely ordered, thus insuring that the phases of scattered light waves is not random. Color production in coherent light scatter depends upon proper phase interactions among light waves scattered from multiple scattering elements. Light waves that are out of phase cancel one another, whereas those in phase enhance one another and are coherently reflected as in constructive interference.

Incoherent light scattering primarily refers to Rayleigh or Tyndall scattering, terms that have been used synonymously over the years although this identity is apparently not the case (Lilienfeld, 2004; Young, 1982). This type of scattering is also described as Mie scattering which is a mathematically correct description of light scatter that more precisely describes the small particle size scattering called Tyndall scattering or Rayleigh scattering (Land, 1972). In any case, incoherent light scattering is the more convenient term which we use here. Well known examples of incoherent light scatter include blue sky, blue smoke, blue ice and blue snow (Bohren, 1987). Incoherent light scatter as the physical cause for blue color has been with us for so long that it has been tacitly accepted even without supporting data (Fox, 1976). As an example, I quote from personal correspondence with Prof. Craig Bohren in 1991: 'Everyone knows, for example, that blue eyes are blue because of Rayleigh scattering. Everyone knows but no one can cite a paper in which this has been demonstrated experimentally'.

Coherent scattering includes several important optical phenomena, notably diffraction or interference. Well-known examples of such reflection include soap-bubbles, the iridescent oil slick on pavement and the bright colors of insects and bird feathers (Fox, 1976; Fox and

Vevers, 1960). Iridescent colors are produced by coherent scattering, but coherent scattering does not always produce iridescent colors (Prum et al., 1998). Among the more important elements involved in the generation of coherent scatter in biological systems are multilayer reflectors such as seen in bird feathers, butterfly scales, and iridophores of lower vertebrates. In the majority of cases, the high reflectance from structures is the result of thin-film or thin-layer interference (Land, 1972). Such systems consist of several alternating layers of materials of high and low refractive index of a thickness comparable with the wavelength of light. While the reflectance at a single interface is high, a much higher reflectivity is produced from a stack of reflective surfaces (Figure 1). At every interface, a certain proportion of incident light is reflected and so with a stack, light reflection of the particular wavelength is additive or constructive. When a variety of perfect conditions for the stack are fulfilled, viz. refractive indices of reflecting layers and intervening space, number of layers and distance between layers, an 'ideal multilayer thin-film' is achieved (Land, 1972). When these conditions are not completely satisfied, the thin-film system is considered to be 'non-ideal'. Such is the case for some systems wherein blue wavelengths are reflected, for example, in a stack of reflecting platelets in an iridophore such as found in the surgeonfish discussed later.

Thanks to elegant and penetrating experimentation on the blue colors of avian and mammalian skin by Prum and Torres (2003a, 2004), we have now been made aware of another important system of coherent light scatter. They have observed that in the dermis of structurally colored skin of a variety of birds and mammals, there exists a thick layer of collagen fibers that are often underlain by melanin. Electron microscopy of the collagen layer revealed the presence of quasi-ordered arrays of parallel collagen fibers. By subjecting electron micrographs of the collagen arrays to two-dimensional Fourier analysis, they observed an appropriate spatial frequency sufficient to produce the observed blue hues by coherent scattering alone (Prum and Torres, 2003b). This discovery that a considerable number of examples of collagen related blue coloration are attributable to coherent light scatter is extremely important. It can no longer be tacitly assumed that blue skin is due to incoherent light scatter (viz. Tyndall blue). The caveat is introduced that, to be sure of the basis of any structural color, the collection of solid data and appropriate analysis are necessary.

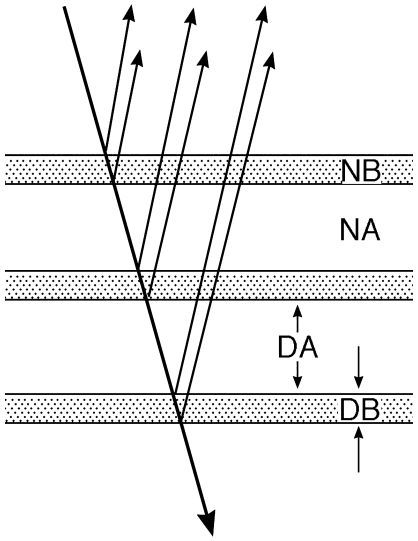

Figure 1. Diagramatic explanation of light rays in a thin-film reflection system. NA and NB, refractive indices of the optically clear and opaque strata in the stack, respectively. DA and DB, actual thicknesses of the optically clear dermis strata, respectively (from Fujii, 1993b).

Blue colors of fishes

Coral reef fish and coherent light scatter

The fluorescent blue colors seen in many fish, for example, the coral reef damselfish and surgeonfish, are the products of coherent light scatter. Given the paucity of blue pigments among vertebrates, the brilliant bluish tints of some coral reef fishes are particularly attractive and have invited investigation of the mechanisms responsible for such coloration. The blue damselfish *Chrysiptera cyanea* attracted early interest (Kasukawa and Oshima, 1987; Kasukawa et al., 1986; Oshima et al., 1985) and studies on it and the blue areas of other coral reef fishes revealed that dermal iridophores are responsible for this color. Indeed, it was concluded that multiple thin-layer interference occurring in stacks of light-reflecting platelets within iridophores provides the basis for the expression of their iridescent blue colors (Fujii, 1993b).

The dermis of the blue damselfish contains a single layer of rounded or ellipsoidal iridophores. The nucleus of each of these cells is apically located and from which reflecting platelets radiate into the cytoplasm. The reflecting platelets are not <5 nm thick (Oshima and Fujii, 1987; Oshima et al., 1985) and thus it was concluded that multilayered thin-film interference of non-ideal status was responsible for the generation of their iridescent blue colors. Some of these damselfish are capable of remarkable color change, a phenomenon attributable to the fact that some of the iridophores are motile (Fujii, 1993b).

Among other beautiful blue fish found in association with coral reefs is the common surgeonfish, *Paracanthurus hepatus*, well known for its beautiful two-tone bluish colors (Goda and Fujii, 1998). As shown in Figure 2A, a dark blue scissor stripe and yellow tail contrast with the sky-blue flank. The dark blue area is dominated by melanophores while the yellow color is due to the presence of xanthophores. Unlike the fluorescent cobalt blue of damselfish, the surgeonfish dis-

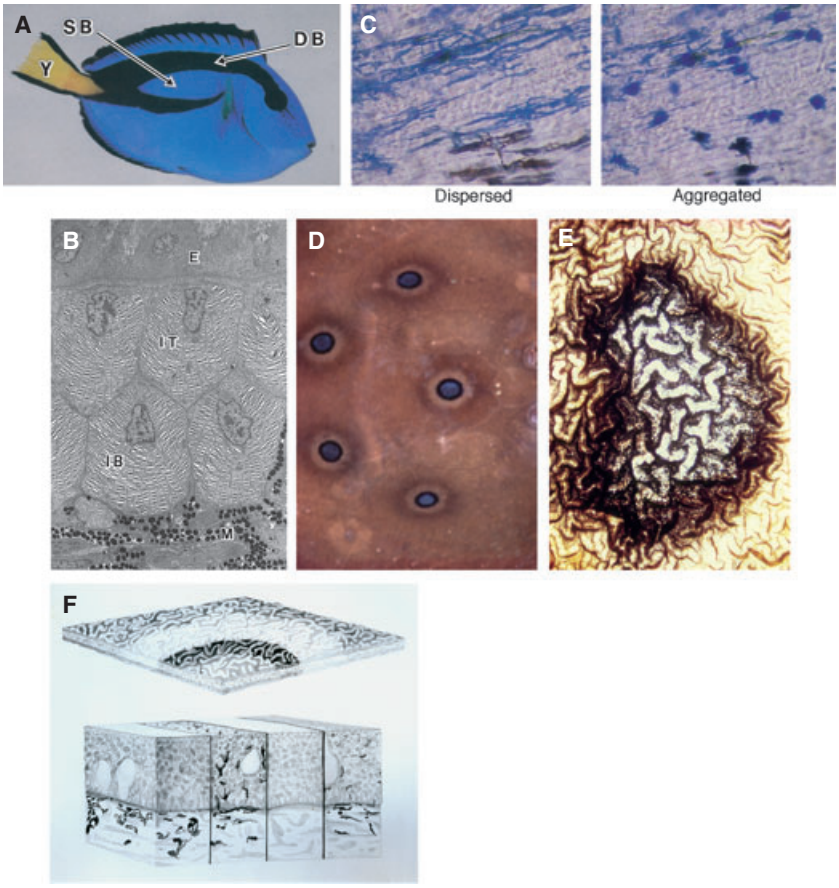

Figure 2. Blue coloration in fish. (A) Photograph of a young surgeonfish, *Paracanthurus hepatus*. The body length of this individual was about 45 mm. The sky blue region in the middle part of the trunk (SB), the dark blue region dorsal to the former (DB) and the yellow portion of the tail fin (Y) were examined (from Goda et al 1994). (B) Low-power electron micrograph of a vertical section across the sky blue part of the dermis of a surgeonfish. Iridophores constitute a double layer, which is lined by a sheet of melanophores. In the iridophores in the upper layer, nuclei are present in the topmost regions within iridophores. In the bottom layer, nuclei are located more centrally. E, epidermis; IB iridophore of the bottom layer; IT, iridophore of the top stratum; M, melanophore. ×3700 (from Goda et al 1994). (C) Cyanophores of the mandarin fish *Synchiropus splendidus* in dispersed or aggregated state as indicated. ×300 (from Goda and Fujii, 1995). (D) Blue spot area on dorsum of *Torpedo ocellata*. (E) A whole mount of *Torpedo ocellata* skin showing a blue spot area viewed under transmitted light. Note that the spot is essentially clear. (F) Artist's reconstruction of blue spots and adjacent region of *Torpedo ocellata*. See text for explanation.

plays a lighter hue that can be more aptly designated as 'sky blue'. Spectral reflectance measurements of the sky-blue portion of the skin show a steep peak at 490 nm, implying that the revelation of that hue might be due to multilayer thin-film interference of the non-ideal type from stacks of very thin reflecting platelets in iridophores of that region, such as occurs in the damselfish. Indeed, the structural organization of the surgeonfish iridophore closely resembles that of damselfish except that rather than being aligned as a monolayer as in the latter, a double layer of iridophores is present in the uppermost part of the dermis (Figure 2B). Likely, the double layer of iridophores explains why the purity of the blue hue of the surgeonfish is rather low in comparison with the vivid cobalt blue tone of the damselfish. Probably, incident light rays are more differentially scattered in the reflecting platelet strata of a double iridophore layer.

Blue chromatophores of callionymid fish

In marked contrast to damselfish and the common surgeonfish whose vivid blue colors are the products of coherent light scatter, shades of blue in two gorgeous species of callionymid fish are produced by a true blue pigment (or pigments) located in a unique blue chromatophore. Studies by Goda and Fujii (1995) on the chromatophores of two callionymid species, the mandarin fish, *S. splendidus*, and the psychedelic fish, *S. picturatus*, revealed the presence of blue dendritic chromatophores that they named cyanophores. Within these dendritic chromatophores, they discovered pigment-containing organelles designated cyanosomes. The organelles are about 0.5 μm in diameter and are composed of fibrous material enclosed by a limiting membrane. Cyanophores of both species respond to various stimulatory cues by the aggregation and dispersion of cyanosomes (Figure 2C). Unfortunately, nothing is known about the nature of the pigments contained in cyanophores other than that in skin specimens treated with alkali, the blue color quickly faded. It is a tragedy that this study has not been carried further, since these observations of a true blue chromatophore containing a true blue pigment are unique among vertebrates.

The blue spots of *Torpedo*

One of the common elasmobranch fishes of the Mediterranean Sea is the electric fish *Torpedo ocellata*. Its light chocolate brown dorsal surface is interrupted by discrete round bright blue spots of about 1 cm in diameter (Figure 2D). In an unpublished study by one of us (JTB) carried out at the Stazione Zoologica di Napoli some 35 yr ago, it was discovered that the blue color of

these spots was produced in a unique way. Observations of the blue spot made on whole skin mounts and viewed with transmitted light revealed that the blue spot itself was actually a clear unpigmented area occupied only by epidermal cells and a few skin glands (Figure 2E). Observations of the whole mounts and skin sections studied by both light and electron microscopy revealed that the blue (or clear) area was circumscribed by a very dark ring and a concentric pale area separating the ring and spot from the homogeneous brown remainder of the dorsum. An artist's reconstruction (Figure 2F) of the four described regions reveals their essential characteristics.

The central blue spot is devoid of epidermal melanophores (-cytes). It possesses a typical basal lamina in association with a well-ordered collagen matrix. In the dermis of this spot area, large dermal melanophores are seen to contain exceedingly large and numerous electron-dense melanosomes. Many collagen bundles are present in the dermis. By contrast, the dark ring area possesses an epidermis laden with epidermal melanophores (-cytes) in association with keratinocytes into which they have deposited large amounts of cytocrine melanin. Melanosomes of these epidermal pigment cells seem to be typically like those of other vertebrates with respect to size and shape (Bagnara and Hadley, 1973). Beneath the basal lamina numerous dermal melanophores with typical melanosomes are present. In the adjacent concentric pale area, no melanophores are found in either the epidermis or the dermis. The brown peripheral area that covers the rest of the dorsal surface contains both epidermal and dermal melanophores. Many fewer epidermal melanophores (-cytes) are present with correspondingly fewer melanosomes deposited in the keratinocytes. Dermal melanophores with more or less normal appearing melanosomes are found just beneath the basal lamina and into the dermis. There seem to be fewer dermal melanophores and less dense melanosomes than in the blue spot or ring areas. It is beyond the scope of this presentation to present details of the ultrastructure of *Torpedo* melanophores. Rather, principal importance is directed to the distribution of melanin and melanophores such that the blue spot area is devoid of epidermal melanin, but is underlain by the presence of strong black pigmentation in the dermis.

In searching for an understanding of how blue color is expressed from a clear skin window overlaying a corresponding deposit of black pigment, the thought arose that incoherent light scatter was responsible for the blue color just as had been suggested for human skin (Fox and Vevers, 1960). At the time, conventional thought was that the blue color of Mongolian spot or other nevi resulted from the scatter or reflection of blue wavelengths of light from the collagen layers of the skin (incoherent scattering) together with an absorption of longer wavelengths of light by melanin deposits beneath the collagen. To test the possibility that such a mechanism is involved in the expression of the blue spots of *Torpedo*, a model system was devised. A small sheet of pressed wood of about the same color as *Torpedo* skin was outfitted with holes of about 1 cm in diameter. This was to simulate the clear area of the skin while the edges of the holes were outlined with black ink to duplicate the black ring. The sheet was than placed in a white tray. In exact correspondence with each of the holes, the surface of the tray was marked with a permanent black ink marker to simulate the very deep melanophores beneath the clear area. To duplicate the incoherent light scatter function of dermal collagen fibers, skim milk, a known incoherent scatterer was poured into the tray beneath the sheet of pressed wood. The model was apparently successful because the holes sitting above black marks and bathed in milk appeared quite blue in color.

It seems most likely then, in referring the model back to the dorsal surface of *Torpedo*, that the blue spots of this fish are produced very much as are the blue colors of some birds and mammals, incoherent scatter of blue wavelengths of light by a diffuse medium at the same time that longer wavelengths between green and red are absorbed by melanin.

Blue colors of amphibians

The integument

Blue as a normal skin color in amphibians is relatively uncommon. Notable exceptions include the Blue-spotted salamander of North America, *Ambystoma lateralis*, and the South American Blue Poison Dart Frog (*Dendrobates azureus*). The common Green Frog, *Rana clamitans* and its larger relative, the Bull Frog, *R. catesbeiana*, are subject to variations wherein large areas of the whole frog appear blue (Berns and Narayan, 1970). More often the blue color is restricted to the head area. Of less common or even transitory occurrence is the manifestation of blue color in species that are normally quite green (Figure 3A). Although it is not immediately obvious, the expression of blue wavelengths from the surface is exceedingly important, in fact essential, for green coloration of many frogs. This fact is perhaps best comprehensible from an examination of the dermal chromatophore unit, the principle means by which most frogs are pigmented (Bagnara et al., 1968).

The dermal chromatophore unit is an association of three chromatophores residing in the dermis just beneath the basal lamina. Uppermost in the unit in contact with the basal lamina is a layer of xanthophores. These yellow pigment cells lie above a layer of iridophores which in turn are underlain by a layer of melanophores that extend finger-like processes upward and over the upper iridophore surface. Iridophores are laden with reflecting platelets, organelles containing layers of crystalline deposits of purines and pteridines (Bagnara et al., 1988). One of the primary functions of the dermal chro-

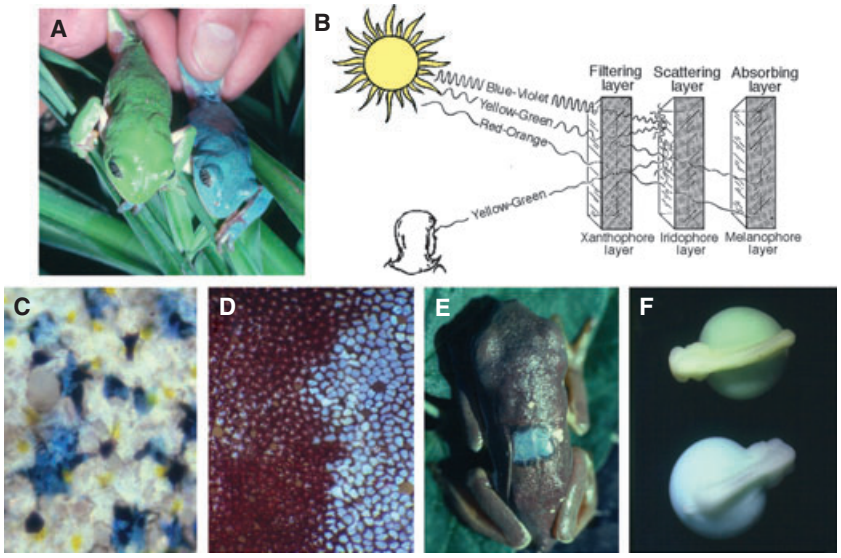

Figure 3. Blue coloration in amphibians. (A) Young sibling males of *P. dacnicolor*. The green individual is normal for the species, while the blue frog is one of only a few that were produced from this spawning. Blue individuals are rare. (B) Diagramatic interpretation for the basis of green coloration in amphibians and other vertebrates. As light strikes the surface of an animal like a frog, short wavelengths of light (blue-violet) are largely absorbed by the filtering xanthophore or yellow pigment layer, the rest are scattered by the iridophore or scattering layer. Long wavelengths (red-orange) largely pass through the filtering and scattering layers of the skin and are absorbed by the melanophore or melanin layer. Intermediate wavelengths (yellow-green) pass through the filtering layer and are scattered from the surface of the iridophore layer and pass back through the filtering layer. Thus, the light reflected from the surface contains a high proportion of yellow-green wavelengths and the animal appears green (from Bagnara and Hadley, 1973). (C) Reflected light photomicrograph of a full thickness wholemount of green skin taken from a leopard frog (*Rana pipiens*). In addition to the yellow xanthophores, note that iridophores are white when they occur singly but are blue when underlain by melanophores. (D) Wholemount of a portion of an alcohol treated skin mount of *P. dacnicolor* that has been manipulated to show function of dermal chromatophore unit. In the darker area, individual iridophores are masked by processes from underlying melanophores, whereas in blue areas light scatter is not affected by overlying xanthophores or melanophore processes, both of which are devoid of pigment (from plate 2.4, pp. 494–495 in Bagnara and Matsumoto, 2006). (E) Recently, transformed froglet of *P. dacnicolor* that had received a dorsal skin graft from a just pre-metamorphic larva. The xanthophores and iridophores of the graft were apparently rejected leaving behind the inert collagen layer to reflect blue light. (F) Two early tailbud embryos derived from clutches of green (normal) and blue (lutein lacking) eggs of *P. dacnicolor*.

matophore unit is to enable rapid color change. Another is to supply a means to achieve green coloration. How this comes about can be understood from recognizing the functions of the individual chromatophore layers. As shown in Figure 3B, the xanthophore layer serves a filtering function, while the iridophore layer, through its content of reflecting platelets that provide surfaces for incoherent (Tyndall) light scatter is responsible for the reflection of shorter wavelengths of light (Bagnara and Hadley, 1973; Bagnara and Matsumoto, 2006). Consistent with most biological systems that reflect blue coloration through incoherent light scatter, the melanophore layer serves to absorb the longer wavelengths of light that are not scattered by overlying iridophores (Figure 3C). Where the three chromatophores are stacked, as in the dermal chromatophore unit, green color is achieved through the absorption of the shorter blue wavelengths of light both from the incident light that strikes the frog and from that which is scattered back from the iridophore layer. The remaining wavelengths that reflect from the frog's surface are dominated by yellow-greens. In the absence of xanthophores or of the yellow pigments that they contain, the surface appears blue. This is shown vividly in Figure 4D, a photograph of a skin mount of *Pachymedusa dacnicolor*, a green frog, which has had its yellow pigments leached away by alcohol, thus depriving the iridophores of their overlying yellow filtering layer. In cases of blue mutants or variants of this and other species of normally green frogs, xanthophores lack yellow pteridine or carotenoid pigments. The blue frog shown in Figure 4A was apparently deficient in xanthine dehydrogenase, a key enzyme in pteridine biosynthesis (Frost, 1978; Frost and Bagnara, 1978). The skin of this species also becomes bluish in cases of carotentoid deficiency wherein xanthophores lack sufficient pigments such as lutein or other acidic xanthophylls (Bagnara and Matsumoto, 2006). As has been the case historically for so many examples of blue coloration of animals (Fox, 1976), we have assumed that blue colors of frogs are attributable to incoherent or Tyndall scatter. In truth, to our knowledge there has never been solid evidence published to confirm this conclusion. Moreover, the number of amphibian species that have been studied from the standpoint of chromatophore ultrastructure and composition is relatively a few. Nevertheless, given all circumstances, we

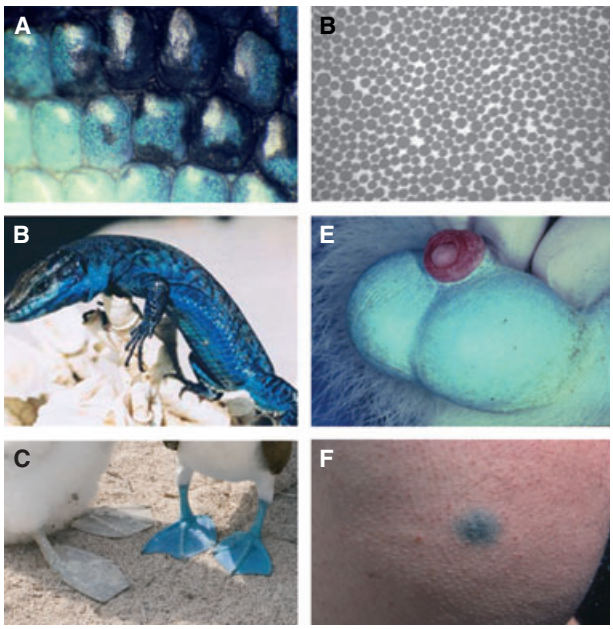

Figure 4. Blue coloration in reptiles, birds and mammals. (A) Reflected light photomicrograph taken *in vivo* of scales in the blue ventral skin of a male *Cophasaurus texanus*. (B) A blue variant of the Italian common lizard, *Podarcis* sicula, from the population inhabiting the Faraglione of Capri, Italy (courtesy of Mr Giuseppe Esposito). (C) The feet of juvenile (left) and adult (right) blue-footed boobies (*Sula nebouxii*) (courtesy of Mr Ryan Sawby). (D) Transmission electron micrograph of a cross section of a collagen array from the blue facial skin of a mandrill, *Mandrillus sphinx*. (courtesy of Dr Richard Prum). (E) Close up of the blue scrotum of a vervet monkey (courtesy of Dr Richard Prum). (F) Human blue nevus.

conclude that there is no alternative other than to continue with the assumption that blue color production in amphibians is a phenomenon of incoherent light scatter from iridophore reflecting platelets.

Lest a preoccupation with iridophores as light scatterers divert attention from other sources of incoherent or even coherent light scatter, it should not be forgotten that the amphibian dermis is laden with collagen masses. It is easy to dismiss the possibility that scatter from such collagen arrays would be obscured by overlying iridophores and xanthophores; however, it appears that it can occur. In a series of unpublished experiments performed by one of us (JTB), reciprocal transplants of dorsal skin were made between late larval and newly metamorphosed stages of *P. dacnicolor*. Postmetamorphic skin grafted on to larvae took and persisted; however, larval skin grafts on froglets were rejected. The rejected grafts lost their chromatophores leaving only their inert collagen components. As can be seen in Figure 4E, the rectangular graft areas became blue in color very likely due to light scatter from the persisting collagen arrays. Whether such scatter plays any role in intact froglets is unknown.

Blue eggs

Of surprise to many, blue coloration of amphibians can also extend to the eggs (ovocytes) of some species. In fact, the case has been made that the ovocyte can be considered as a kind of pigment cell at least in an homologous sense (Bagnara, 1985). The best evidence to support this contention comes from studies of the Mexican leaf frog, *P. dacnicolor*, and here we review the basis for the pigmentation of the eggs of this species. Consistent with its common name, this frog lays its eggs in foliage above pools of water. The eggs are normally green in color and blend in cryptically with the surrounding vegetation. Just as with the integument whose green coloration is based upon the presence of both blue and yellow color sources, leaf frog eggs are green (Figure 3F) because of the presence of both blue and yellow constituents. In this case, the elements are actual pigments; the blue, the hemoglobin derivative biliverdin IXα and the yellow, the xanthophyll pigment, lutein (Marinetti and Bagnara, 1982, 1983). During ovogenesis, both pigments are transported from the liver to the ovary and deposited in the yolk (vitellus). The degree of greenness of the egg is dependent upon the relative amounts of the two pigments. Female frogs fed a low carotenoid diet during vitellogenesis are lutein deficient and the eggs become quite blue (Figure 3F). On the other hand, in the absence of biliverdin IXα, as in hemoglobin deficient mutants, the eggs are yellow. Hemoglobin deficient mutants on a low carotenoid diet produce white eggs. Thus, just as with the integument, the appropriate balance between blue and yellow elements determines the relative greenness of the egg. In keeping with the theme of this review, the importance of blue coloration is again displayed.

Blue colors of reptiles

The bright skin colors of lizards are produced from the interactions of dermal chromatophores. The work of Rohrlich (1974) and Rohrlich and Porter (1972) focused on the ultrastructure of iridophores and of how their interaction with dermal xanthophores and melanophores produce the brown to green skin color in the lizard *Anolis carolinensis*. In addition to their detailed descriptions of iridophore reflecting platelets, they found that isolated iridophores appear blue-green under reflected light, and red when viewed with transmitted light. They made the important distinction that the iridophores underlain with melanophores were 'intensely' blue-green, presumably due to absorption of longer wavelengths by the melanin, and the iridophores associated with yellow xanthophores appeared green. In light of our current recognition of how arrays of extracellular fibers generate structural colors, it is interesting to note that Rohrlich and Porter (1972) also described an intracellular 'filament system' in iridophores arranged as a lattice between layers of reflecting platelets. Other species of *Anolis*, such as *A. allisoni* and *A. gorgonae*, that exhibit

brilliant blue skin would seem ideal for further studies to elucidate the basis of blue coloration. Unfortunately, there are relatively few studies of the pigment cell biology of other *Anolis* (Macedonia et al., 2000; Moermond, 1978).

The blue abdominal skin in some lizards is a sexually dimorphic trait that is more pronounced in males (Figure 4A). Quinn and Hews (2003) described how elevated testosterone levels induce both dermal melanization and blue abdominal skin. Exogenous testosterone not only enhanced the blue color of male abdominal skin, but also produced male-like blue abdomens in females. The most obvious effect of the testosterone on pigmentation was significantly increased dermal melanization under iridophores in the blue skin, which once again implicates melanin as an important absorber of wavelengths other than blue.

Although testosterone can clearly produce sexually dimorphic blue coloration, all individuals of some populations of the Italian common lizard, *Podarcis sicula*, are an intense blue color (Figure 4B). Dr Dominico Fulgione has observed that both males and females are blue throughout the annual cycle (personal communication). These lizard populations are restricted to rocky cliffs on small islands on the west coast of Italy, not far from Naples (the Faraglioni Cliffs of Capri and the islet Licosa just south of Paestum). To date, there has been no description of the physical or physiological basis for the blue color of these lizards.

Morrison (1995) demonstrated how mathematical models can be predictive of observed lizard skin colors when the size, shape and spacing of the crystalline reflecting platelets of iridophores are accounted for. In contrast to the thin, elongated reflecting platelets of fish iridophores, lizard iridophores tend to have thicker and more square or rectangular profiles. Morrison et al. (1995) examined the blue ventral belly patches and throat skin of three species of the spiny lizard, *Sceloporus*, and found that as the size of iridophore reflecting platelets increased, skin color transitioned from dark blue to light blue to green to yellow to orange.

We owe a debt of gratitude to herpetologists who collect and herpetoculturists who breed and maintain brightly colored variants of many species (Bechtel, 1995). Among the most prized are specimens that display blue coloration and these individuals are often described as 'axanthic'. In species that are normally green, we generally assume a lack of yellow pigmentation causes the color variation, but we must not ignore the possibility that coherent blue light scatter may be the cause of a blue phenotype. An example of this may come from the examination of a blue mid-dorsal stripe on an individual garter snake, of the genus *Thamnophis* (Bagnara et al., 1978). In some populations, the mid-dorsal stripe is normally red, due to the presence of erythrophores containing well-formed pterinosomes and pteridine pigments. In the variant blue stripe, the pteridine content was greatly reduced and what appeared to be pterinosomes were rounded organelles possessing unorganized areas of fibers, not unlike the unusual cyanosomes of the cyanophores of the callionymid fish mentioned earlier. Reflecting platelets of many iridophores in the blue stripe were almost completely collapsed. In the absence of red pigments, blue wavelengths of light reflected from this anomalous area could have produced the blue stripe. The defective iridophores may have provided a surface capable of reflecting blue light; however, a contribution by dermal collagen arrays in this area cannot be excluded.

Much of the functional significance of conspicuous blue coloration is attributed to eliciting a behavioral response in the observer (Parker, 1998). Studies of behavior and sexual selection identify dimorphic color patterns, including blue abdominal and throat skin as a vitally important trait (Cooper and Burns, 1987; Hews and Quinn, 2003). The blue tails common among many types of juvenile lizards are thought to lend protection by distracting predators' attention to the expendable appendage (Hawlena et al., 2006). In addition to blue skin at least one reptile, the blue tongue skink (*Tiliqua scincoides*), continues the theme on its lingual epithelium which is conspicuously displayed when the lizard is disturbed by potential predators. Although it would provide a fascinating comparison to blue integument, it appears that little is known of the microscopic structure of the blue tongue.

Blue colors of birds

The striking diversity of avian coloration is due to the remarkable morphologies of the integumental structures of birds (Finger et al., 1992). The blue color of feathers, skin, scales and bills are structurally derived through various combinations of epidermal keratinocytes, epidermal pigment cells and dermal connective tissue fibers.

The keratinocytes that develop into feathers produce repeatedly dividing branches from a main shaft, or rachis. Barbs branch off of the rachis and smaller barbules branch off of the barbs. The structural blue colors of feathers are produced by the barbs and barbules. The structural blue colors may be iridescent or non-iridescent. Iridescent structural colors tend to change in appearance with changes in the angle of observation or illumination while non-iridescent structural colors are consistent regardless of viewing geometry (Osorio and Ham, 2002). Feather barbules tend to be iridescent while the feather barbs usually produce non-iridescent structural color (Doucet et al., 2006).

Non-iridescent feather color is normally produced by coherent scattering of light by ordered, alternating layers of keratin and air that form a spongy medulla within feather barbs (Dyck, 1976; Prum, 2006; Prum et al., 1999a,b). The medulla is underlain by a layer of melanin granules. In this system, the melanin absorbs wavelengths other than the blue light that is coherently scat-

tered by the spongy medulla. The role of the melanin granules in producing this type of non-iridescent structural blue was demonstrated by Shawkey and Hill (2006) who described the feather structure of an amelanotic Steller's jay (*Cyanocitta stelleri*). This white bird lacked melanin in its feathers as well as the typical blue feather color of this species. Spectrographic comparison of the anomalous white feathers and normal blue feathers revealed that while both white and blue feathers reflected blue light (presumably from the layered medulla), the melanin in the blue feather absorbed incoherently scattered white light resulting in a more purely blue appearing feather.

Iridescent blue colors of feather barbules are also produced by coherent light scatter from layers of keratin, melanin and air. However, a wide range of iridescent colors are generated by altering the thickness, shape and spacing of the materials in each layer (Doucet et al., 2006). Differences in the shape of melanosomes appears to play a significant role in the iridescent color difference between, for example, the gorgets of hummingbirds (Greenwalt et al., 1960) and peacock (*Pavo muticus*) tail feathers (Zi et al., 2003).

The exposed skin of birds may also be bright blue. Prum et al. (1994) were the first to describe a mechanism for the structural colors of bird skin and their analysis using electron microscopy revealed that non-iridescent green and blue skin colors are produced by coherent scattering from hexagonally organized arrays of dermal collagen fibers. This is the color mechanism for the blue skin of the head, legs and feet of a variety of birds including the aptly-named blue-footed booby (*Sula nebouxii*) (Prum and Torres, 2003a; Prum et al., 1999a,b). It is interesting to note that that the feet of juvenile blue-footed boobies are gray and only develop the characteristic bright blue color as the birds mature (Figure 4C).

The structural color produced by the highly ordered, crystal-like arrangement of dermal collagen fibers is analogous to the highly ordered crystalline reflecting platelets of amphibian and reptile dermal chromatophores. In addition, just as the blue-green color reflected by reptilian iridophores is accentuated by underlying melanin, the blue to green reflecting dermal collagen fibers in bird skin are underlain by a layer of melanocytes (Prum et al., 1999a,b). However, blue color can be theoretically achieved in some tissues without melanin simply from the spacing of nanostructures such as keratin granules or collagen fibrils.

Although the color of avian skin and feathers is often attributed to carotenoids, it should be noted that green and yellow-colored bird skin, in addition to blue, can be structural colors produced by coherent light scatter from dermal collagen arrays without contribution from carotenoids or underlying melanin (Prum and Torres, 2003a).

Bird bills (ramphotheca) can also exhibit non-iridescent structural color. Bill color is stable all year in most birds, but during the breeding season, the bill of the male ruddy duck (*Oxyura jamaicensis*) changes from black to blue. The color is produced from coherent light scatter from parallel, quasi-ordered arrays of dermal collagen fibers (Prum and Torres, 2003a).

The pigmentation of the brightly colored iris of several avian species is remarkably similar to the brightly colored dermis of fish, amphibians and reptiles (Ferris and Bagnara, 1972). Examples of yellow, gold and red irides have been shown to be produced in some birds by reflecting pigment cells that are functionally and structurally analogous to the dermal iridophores (Oliphant, 1987; Oliphant et al., 1992; Tillotson and Oliphant, 1990). To our knowledge, none of the species examined to date have blue irides. It would be fascinating to learn if the brilliant blue irides of the satin bowerbird (*Ptilonorhyncus violaceus*) are also produced by the iridophore-like cells of 'lower' vertebrates or if the avian/mammalian mechanism of ordered collagen fiber arrays are employed.

Blue colors of mammals

In marked contrast to lower vertebrate taxa, fishes, amphibians, reptiles, and birds, blue as a major color among mammals suffers by comparison. Nevertheless, mammals have better perfected coherent light scatter from dermal collagen arrays and thus have perhaps surpassed their lower vertebrate counterparts (Prum and Torres, 2004). For many years, it was considered that the prominent blue colors of some primates such as the mandrill (*Mandrillus sphinx*) that possesses spectacular blue facial skin and a blue rump are the product of incoherent light scatter (Fox, 1976). This conclusion was generally accepted to be true although it was challenged, notably by Findlay (1970) who held that light scatter was unimportant in the generation of blue colors of the skin. Rather, he considered blues to arise from an optical relationship between collagen and melanin leading to a 'subtractive color mixing' in the dermis. His conclusions were derived from observations on the skin of patients including two cases of Bantus with Mongolian spot and vervet monkeys (*Cercopithecus aethops*) which possess a remarkably blue scrotum. An analysis of his investigation by Prof. C. Bohren (personal communication) and by Prum and Torres (2004) cast serious doubt on Findlay's conclusion. Therefore, it appears that incoherent light scatter can no longer be considered the principal cause for blue skin color in mammals. Rather, it seems that this color is more likely attributable to coherent light scatter. Apparently, this idea was first advanced by Oettle (1958) who proposed from experiments on the vervet monkey that dermal collagen was the source of coherent light scatter responsible for the blue scrotal color. Oettle's work was given short shrift by Findlay (1970) and, as was pointed out by Prum and Torres (2004), Oettle's cogent observations attracted relatively little attention. However, in keeping with their

earlier work on the blue color of avian skin, they were able to show that in the dermis of the mandrill, vervet monkey, and other mammals, collagen arrays are indeed the source of the coherent light scatter responsible for the blue color (Prum and Torres, 2004). Through the use of transmission electron microscopy (TEM) of cross sections of dermal collagen arrays (Figure 4B) it was possible to make measurements of fiber diameter and distances between fiber centers. The analysis of these data together with spectral reflectance from the skin of corresponding areas allowed for an understanding of how particular blues are derived. As a result they were able to show that the frequent association of melanin deep in the dermis with coherent scatter was not necessarily the case in the mandrill. Here, facial blue skin is not underlain by melanin and constructive interference alone is able to produce blue coloration. On the other hand, in the blue rump skin, melanin is present beneath the collagen arrays. Similarly, melanin is found to underlay the collagen arrays in the blue scrotal skin of the vervet monkey.

The question arises, of course, of what is the function of blue coloration in mammals. Numerous investigations have considered this question and one prevailing general conclusion is that strongly visible colors function in intraspecific communication. Scrotal color seems to be of particular significance as a signal of social status. Very likely, a strongly colored blue scrotum is profoundly influential in mate choice by females. A fascinating discussion of the role of the blue scrotum in vervet monkeys is presented by Struhsaker (1967). He describes the 'red, white and blue' display of dominant males, based upon the presence of a red penis (due to capillary blood), a white belly, and the blue scrotum (Figure 4E).

The evolution of the collagen arrays responsible for the blue coloration of mammals is an intriguing consideration that has been addressed by Prum and Torres (2004). They investigated the structure and biophysics of two primate species, the mandrill (*Mandrillus sphinx*) and the vervet monkey (*Cercopithecus aethops*); and two species of marsupials, the mouse opossum (*Marmesa mexicana*) and the wooly opossum (*Calurompus derbianus*). In addition they noted that the presence of structural colors in an array of close relatives of both mandrill and vervet monkey and have concluded that structurally colored skin has evolved at least twice within the old world primates. Similarly, they have concluded from the presence of blue scrotum skin in various marsupial relatives that structurally colored skin must have evolved two or more times in the marsupials.

It is important that dermatologists and others interested in hypermelanosis of human skin begin to pay attention to the importance of dermal collagen arrays in the expression of blue colors in a variety of dermal hypermelanoses (Figure 4F), especially Mongolian spots, nevus of Ota, nevus of Ito, among others. For a concise presentation of acquired and dermal hypermelanosis, consult chapter 52, pp. 1103–1119 of the exhaustive compendium on the pigmentary system by Nordlund et al., 2006. There are a variety of parameters particular to each of these lesions, but the important thing that they have in common, other than their blue color, is that each is circumscribed by a deposition of melanin essentially beneath the dermal collagen arrays. Very often the melanin is located in dermal melanocytes that did not migrate into the epidermis during embryogenesis. The ultimate position of the melanocytes or melanin determines the color of the respective macules. For instance, the nevus of Ito, located in the upper arm or shoulder region, typically has flat blue-black or slaty macules intermingled with small, flat brown spots. These brown spots result from localized epidermal hyperpigmentation, whereas the blue-black or slaty macules are caused by the presence of dermal melanocytes. Similarly, the nevus of Ota, a benign melanocytosis of the facial area innervated by branches of the trigeminal nerve, has been classified into five histological types that correlate well with the visible color of the nevus. More superficial lesions are brownish, whereas deeper types are more increasingly bluish. In the absence of precise data relating to measurements of dermal collagen arrays in correlation with spectrophotometry, it can be only speculated that the color of these human blue colored macules or nevi is based upon a system of coherently scattering dermal collagen arrays in conjunction with underlying melanin, although the perception of their relative blueness may be influenced by the color around the skin (Reisfeld, 2000).

Discussion

Rationale for the compilation of this review of blue colors among vertebrates comes largely from the fact that in the area of pigmentation, investigators working at one end of the vertebrate scale seem relatively unaware of the work that goes on at the other end. As a result, scientists on each side may not realize just how much they have in common. One of these groups of common interest deals with the nature of and the physical basis for blue coloration in vertebrates. Given the rarity of true blue pigments, major focus is placed upon structural coloration and the light scatter upon which it is based; namely, incoherent light scatter and coherent light scatter.

The varying sources for these types of scatter are fascinating in that both incoherent and coherent light scatter may emanate from either intracellular or extracellular surfaces. Among the cold-blooded vertebrates, fishes, amphibians and reptiles, blue colors are reflected both incoherently and coherently from the reflecting platelets contained in iridophores. The best known example of the latter occurs in the coral reef fishes where it was shown that multilayer thin-film interference of the non-ideal type is responsible for the bright blue and often

iridescent colors (Fujii, 1993b). It has been suggested that the same process of coherent light scatter provides the basis for blue color in some lizards (Morrison, 1995), but this has not been as well documented as it has been for fishes. For the most part, it is assumed that light scatter from iridophore reflecting platelets of many fishes, amphibians, and lizards is of the incoherent type. However, blues in some fishes and reptiles are often iridescent, implying the presence of coherent light scatter.

The degree to which light scatter from extra-cellular sources is responsible for or contributes to blue coloration in lower vertebrates is not known. However, because of the clear demonstration that collagen arrays in the dermis of birds (Prum and Torres, 2003a) and mammals (Prum and Torres, 2004) coherently scatter light to provide the basis for their blue coloration, is seems likely that the same may hold for at least some lower vertebrates. Unfortunately, without any firm data about the possibility of incoherent or coherent light scatter from dermal collagen arrays of lower vertebrates, it can be only speculated that such occurs. One argument that extra-cellular light scatter is not a significant factor for blue color expression in lower vertebrates stems from the fact that iridophore layers for the most part lay above the collagen arrays and would thus block the passage of reflected blues. On the other hand, a good lower vertebrate candidate for speculation that coherent light scatter from dermal collagen arrays is responsible for blue color is *Torpedo* where blue spots are expressed on the dorsum. Here, the structural resemblance of the blue spot areas to blue nevi of human skin is remarkable.

With the likelihood that the capacity for coherent or incoherent light scatter from collagen arrays exists throughout the vertebrates, thoughts about the evolution of this function arise. Unfortunately, specific knowledge about these arrays among the vertebrates is not generally known and thus we are relegated to the realm of speculation. However, given the relatively conservative nature of collagen molecules among vertebrates, it seems likely that intertaxonomic differences in collagen per se are not great and possibly would not provide much evolutionary insight. However, we must remember that collagen fibrils do not stand alone; rather, when speaking of collagen in a biological sense we are dealing with a collagen aggregation system that contains other supporting molecules between the fibers. Among these are mucopolysaccharides such as hyaluronic acid, a rather hydroscopic agent capable of swelling and as a consequence capable of altering the reflection pattern of light from the collagen arrays (Chvapil, 1967). This and other factors would be important in considering the evolution of coherent and incoherent collagen arrays in the scatter of light in vertebrates.

Perhaps the greatest void in our knowledge of the basis of blue colors among vertebrates is the lack of new knowledge about cyanophores and their constituent pigment or pigments. There can be no doubt that Goda and Fujii (1995) discovered a true pigment, but it seems absurd that no new knowledge has been forthcoming. It seems equally absurd to consider that among all vertebrate species this unique pigment has been found in only two species. Even if this unknown pigment is not present in any other vertebrate Class, it surely must be present in some other piscine taxa. This lack of knowledge begs further investigation at least on the identity of the pigment, the organelle in which it is contained, and its distribution among piscine and other vertebrate species.

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