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Molecular basis for color vision *

Tôru Yoshizawa

Department of Information Systems Engineering, Faculty of Engineering, Osaka Sangyo University, 3-1-1, Nakagaito, Daito-shi, Osaka 574, Japan

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Abstract

Amino acid sequences of four kinds of chicken cone pigments and two kinds of nocturnal gecko visual pigment were determined. Calculations of amino acid identities indicate that gecko pigments should be cone pigments. A phylogenetic tree of visual pigments constructed demonstrated that cone pigments evolved earlier than rod pigments (rhodopsins), indicating that daylight vision including color vision appeared earlier than twilight vision. The divergence of cone pigments to rhodopsins would be caused by replacing basic amino acid residues to acidic ones according to net charge calculations. A comparison between chicken rhodopsin and cone pigments (chicken green and red) displayed that the cone pigments are faster in regeneration from 11-cis retinal and opsin, faster in formation of meta II-intermediate and shorter in lifetime of meta II-intermediate than rhodopsin. These facts would partly explain the rapid dark adaptation, the rapid light response and the low photosensitivity of cones compared with rods. In comparison with di- and tri-chromatic color visions, chicken tetra-chromatic vision was discussed on the basis of both absorption spectra of cone pigments and filtering effect of oil droplets.

Key words: Rhodopsin; Iodopsin; Cone pigments; Oil droplet; Evolution of visual pigments; Color vision

1. Introduction

Most vertebrates have two kinds of visual systems, namely twilight (or scotopic) vision and daylight (or photopic) vision. There are two kinds of visual cells in the retinas: one is rods responsible for twilight vision which can discriminate among light intensities under dim light and the other is cones responsible for daylight vision. The rod contains rhodopsin as a visual pigment, while the cone contains a cone pigment like iodopsin. If an animal has two or more types of cones, each of which has an own cone pigment different in absorption spectrum, it may recognize colors. In case of humans, there are three kinds of cone pigments, that is, red, green and blue sensitive pigments, which are called human red, green and blue, respectively (see Fig. 1B).

The molecular mechanism of primary process of vision has exclusively been studied on twilight vision so far. Now the main path of the transduction process in the rod from absorption of a photon by a rhodopsin molecule to generation of

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the receptor potential has been elucidated. On the other hand, the study on daylight vision including color vision, which has a more close connection to our daily life than twilight vision, has been prevented because of difficulties of isolation of cone pigments from retinas.

2. Cone pigments and molecular evolution

In 1955 Wald et al. [1] reported that a chicken retina has two kinds of visual pigments, that is, rhodopsin as a rod pigment and iodopsin as a cone pigment. Both the pigments have the same chromophore, 11-cis retinal, but are different in protein-moiety, opsin, from each other. Several years ago, we developed a new method for isolating chicken visual pigments [2], in which CHAPS (0.6%) supplemented with phosphatidylcholine (0.8 mg/ml) and glycerol (2%) was used as a solubilizer together with a series of column chromatographies (ConA Sephalose affinity, DEAE-Sepharose and CM-Sephalose), and succeeded to purify one type of rod pigment (rhodopsin) and four types of cone pigments, that is, chicken green, chicken red (also called iodopsin), chicken blue and chicken violet, the latter of which was obtained as a mixture with chicken blue.

The spectra of chicken cone pigments are shown in Fig. 1A, which are regularly distributed on the wavelength scale in comparison with human cone pigments (Fig. 1B). Furthermore, human has no pigment corresponding to chicken blue, and human red and green are close in absorption maximum to each other in comparison with chicken ones. Now questions arise why human has only three types of cone pigments and why their spectra are irregularly distributed on the wavelength scale. These questions must be discussed in evolutional aspect of visual pigments.

Then, we determined amino acid sequences of the four types of chicken cone pigments [6,7], using the purified cone pigment preparations and cDNA analyses. Furthermore, we investigated nocturnal gecko visual pigments for the following reasons. Nocturnal geckos (lizards) have only rod-shaped visual cells, while most diurnal lizards have only cone-shaped visual cells [8]. In lizard

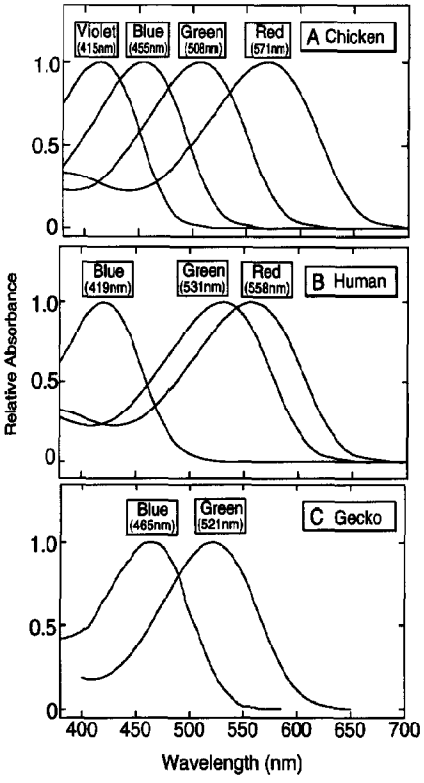

Fig. 1. Absorption spectra of (A) chicken [2,3] (B) human [3,4] and (C) gecko ([5], Kojima, unpublished observations) cone pigments.

order (Lacertilia) there are many grades of intermediate shaped visual cells between rod-shaped visual cells of nocturnal gecko and cone-shaped ones of diurnal lizard. According to a transmutation theory proposed by Walls [8], rod-shaped visual cells of nocturnal geckos morphologically changed from cone-shaped ones of diurnal lizards in forming a nocturnal habit, though the diurnal lizards had lost the rods in making a diurnal habit. Nevertheless, the gecko rods have two types of visual pigments, i.e. gecko blue and green (Fig. 1C). Gecko blue is a rhodopsin-like pigment, while gecko green is an iodopsin-like pigment in physical and chemical properties according to Crescitelli [9]. If the gecko has any cone pigment in the rod, this would give a good support to the transmutation theory.

Then we calculated amino acid identities between the visual pigments of gecko and other vertebrates after determination of amino acid sequences [5]. The results obtained showed that gecko green is very close to iodopsin (83.7% identity). Similarly, gecko blue is close to rhodopsins (64.3–75.5% identity) but it is closest to chicken green (80.3% identity) [3,5], indicating that gecko blue should be a cone pigment. Therefore, both the pigments are cone-type pigments located in the rod-shaped visual cells. Thus gecko visual cells could be called cones rather than rods. The present result not only supports the transmutation theory strongly but also suggests the differentiation of cones to rod-shaped visual cells in the course of evolution. Our recent immunohistochemical observation of chicken retinas in the course of development, demonstrated that the cones appeared ontogenetically earlier than the rod [10].

On the basis of the amino acid identities of all the vertebrate visual pigments which have been analyzed so far, a phylogenetic tree of vertebrate visual pigments was constructed. As shown in Fig. 2, an ancestral visual pigment first diverged to vertebrate and invertebrate visual pigments, and then the ancestral vertebrate visual pigment gene evolved into four Groups L, S, M1 and M2, each of which is composed of pigments similar in wavelength of absorption maximum. Finally, rhodopsin group (Group Rh) derived from Group M2. Thus, the ancestral visual pigment is close to a cone-type pigment rather than rod-type pigment, so that one may suppose that invertebrate visual pigments would be similar to cone-type pigments rather than rod-type pigments.

As already described, cone pigments had evolved earlier than rhodopsin, so that the daylight vision would appear earlier than the twilight vision in the course of evolution. Precisely speak-

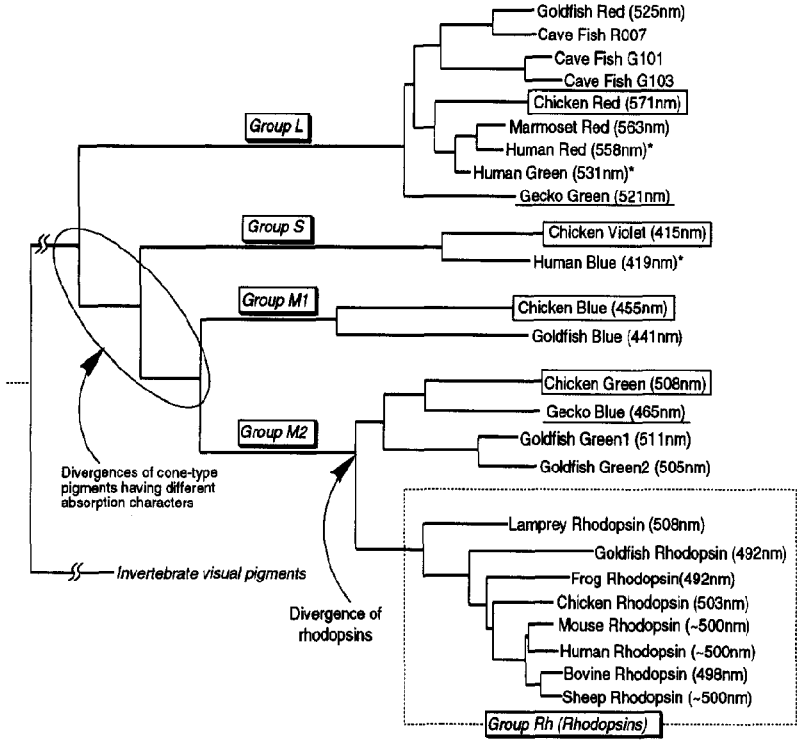

Fig. 2. A phylogenetic tree of visual pigments. For constructing the tree, Neighbor joining method [11] was used. (Modified from Okano et al. [3,7].)

ing, animals in the first stage of the evolution as seen in the phylogenetic tree (Fig. 2), have only one type of cone pigment, so that the animals should be in monochromatism, that is complete color blind. Animals in the second stage have two types of cone pigments; one is belonging to Group L and the other to a common ancestor pigment of Groups S, M1 and M2. These animals should be in dichromatism, i.e. red or green color blindness. In the third stage, animals have three cone pigments belonging to Groups L and S with a common ancestor pigment of Groups M1 and M2, so that they are in trichromatism. In the fourth stage, animals have four types of cone pigments belonging to Groups L, S, M1 and M2, so that they are in tetrachromatism. Thus the animals in the later stages would have excellent ability for color discrimination. It is needless to say that these stories are based on the assumption that light-induced responses of visual cells containing these cone pigments would be integrated into color discrimination. Thus, one may conclude that animals have acquired color vision earlier than twilight vision. Figuratively speaking, animals would have gained a color film earlier than a highly photo-sensitive film in the course of evolution.

According to recent microspectrophotometric observations of retinas, some kinds of fishes [12] and reptiles [13] have four types of cone pigments like chicken. The nocturnal gecko, however, has only two types of cone pigments, because the cone pigments belonging to Groups S and M1 disappeared in forming the nocturnal habit [8]. The similar but slightly different degeneration of cone pigments may be seen in mammals. In fact, most of them are color blind, and only an insectivore, a rodent and primates are confirmed to have color vision [13].

It has been supposed that mammals had evolved from an ancestor of reptile. For escaping from falling a victim to large reptiles like dinosaurs, an ancestor of mammals would be small in size and have nocturnal habit. Then it would have diverged to have a highly sensitive twilight vision owing to rhodopsin evolved in group M2. Instead, the cone pigments belonging to groups M1 and M2 had degenerated. Therefore, the ancestors of mammals would be in dichromatism, having two types of cone pigments belonging to presumably Groups L and S. In fact, some kinds of insectivore, rodent and new world monkey have been proved to have two types of cone pigments, so that the latter would be red–green blind [14]. An old world monkey, however, has normal color vision like human. As shown in the phylogenetic tree, an ancestral human cone pigment belonging to group L diverged into two cone pigments on replacement of several amino acid residues [15], resulting in human green and red (94.9% identity [3]). Thus, human is in trichromatism. This would be the reason why human has no pigment corresponding to chicken blue, and why both the red and green sensitive pigments are close in absorption maximum to each other.

Now, we have another question: what is the essential difference in structure between rod and cone pigments. Various physical and chemical properties of visual pigments were examined. The only difference we have found, is the isoelectric point; chicken and mammal rhodopsins have their isoelectric points at about 6.0, indicating acidic or neutral protein, while all the cone pigments including gecko visual pigments have more than 9.4, indicating basic proteins [7]. A rhodopsin of lamprey [16], one of the lowest vertebrate, has an isoelectric point at about 8.7, which is just between the isoelectric points of rod and cone pigments [7]. Thus, an ancestor of cone pigments as a basic protein had been changed over lamprey rhodopsin to a high vertebrate rhodopsin as an acidic protein by replacing some of the basic amino acid residues into acidic ones in the course of evolution. At present time, one cannot explain the difference in physiological function between rods and cones in terms of isoelectric point.

3. Physiological functions of cone pigments

Notable differences in physiological function between rods and cones have been observed in light response, photosensitivity and dark-adaptation have been known. In order to account for these differences in terms of visual pigments,

bovine or chicken rhodopsin and chicken iodopsin have been compared with each other so far. As seen in Fig. 2, rhodopsins and iodopsin are apart in the phylogenetic tree, and also different in absorption maximum from each other. Chicken green, however, is very close to chicken rhodopsin in primary structure and spectrum. Thus both the pigments should be compared with each other for accounting for the differences in physiological function between rod and cone.

Now, we have measured the following four kinds of reactions for comparison with respective physiological functions [17]. (1) The regeneration rate of visual pigment from 11-cis retinal and opsin for the dark adaptation. (2) The photosensitivity of isomerization of visual pigment to the primary intermediate for the photochemical sensitivity. (3) The formation rate of meta II-intermediate for the light response. (4) The lifetime of meta II-intermediate for the signal amplification.

As a measure of the regeneration rate, the half time of the full regeneration of chicken rhodopsin, chicken green or iodopsin was estimated: 4 h in rhodopsin, 3 min in chicken green and 1 min in iodospin. From these results, one can conclude that the rapid dark adaptation of cones compared with rods would be explained in terms of the rapid regeneration of cone pigments in some extent.

Next, the photosensitivities of chicken green and chicken rhodopsin were compared by measuring the time course of formation of batho-intermediate at liquid nitrogen temperatures. Both the pigments were identical in time course to each other. Since iodopsin is almost identical in photosensitivity to chicken rhodopsin [18], one can conclude that rhodopsin and cone pigments are identical in photosensitivity to each other. Therefore, rod and cone should be identical in photochemical photosensitivity to each other. Accordingly, one cannot explain the difference of sensitivity between rod and cone in terms of the primary photochemical reaction.

It is generally accepted that meta II-intermediate is a physiologically active intermediate which can trigger the visual transduction process [19], so that the thermal behaviors of meta-intermediates of chicken green, red and rhodopsin were investigated to get some molecular clues as to the light response and the signal amplification of visual cells. Using pico- and nano-second laser photolyses, we have measured time constants of formation and decay of intermediates of chicken green, red and rhodopsin at physiological temperatures [17,20,21].

The results obtained showed that these three visual pigments are similar in rate of the primary steps to each other, but different in later steps from each other, i.e. the formation of meta II-intermediate is 200 ms in chicken green and 800 ms in chicken rhodopsin. This fact should be an indication of the rapid response of cone. It is interesting that this indication was detected even in the first step of the visual transduction process.

In addition, it was found that the decay rate of meta II chicken green was 23 times faster than that of meta II-rhodopsin. In other words, meta II-chicken green is shorter in lifetime than meta II-rhodopsin. Since the GDP–GTP exchange reaction catalyzed by meta II-intermediate is the first step of the amplification in the visual transduction system, the longer the lifetime, the larger the extent of amplification. Thus the shorter life time of meta II-chicken green would explain the lower extent of amplification in cones compared to that in rods.

4. Color discrimination and physiological function of oil droplets

A chicken retina has five types of colored oil droplets, five types of visual pigments and six types of visual cells, that is, one type of rod, four types of single cones and one type of double cone composed of principal and accessory members. All these cones have an oil droplet in their inner segments except for the accessory member of the double cone. Since the rod has no oil droplet, one can easily distinguish between rods and cones by means of a light microscope.

On the basis of immunohistochemical observations of visual cells, counting the population of each oil droplet in the retina and estimating the content of each visual pigment in the extract, the pairing of visual pigment and oil droplet in each

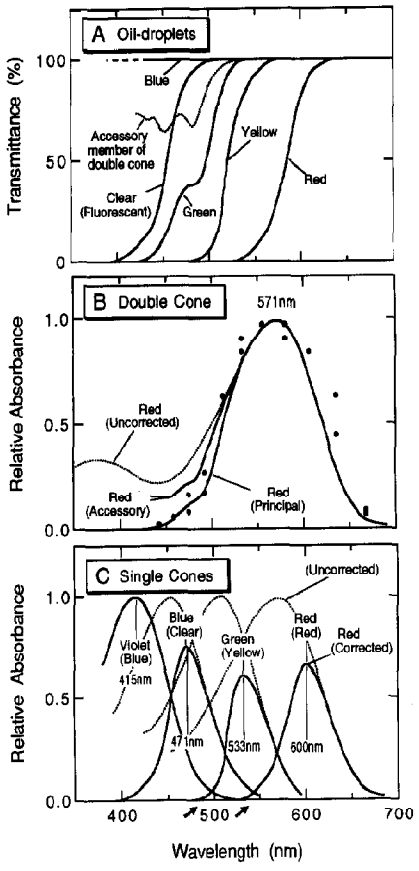

Fig. 3. Chicken photopic sensitivity curves and spectra of chicken cone pigments corrected for filtering effects of the oil droplets. (A) Transmittance curves of chicken oil droplets. The curves were recorded by Bowmaker and Knowles [23]. (B) Sensitivity curves of chicken double cone calculated from the absorption spectrum of iodopsin. Closed circles: A chicken photopic sensitivity curve recorded electro-physiologically from chicken retinas by Wortel et al. [24]. Dotted line (Red, uncollected): spectrum of iodopsin. Solid line (Red, principal): spectrum of iodopsin corrected by the transmittance curve of green oil droplet in the principal member (Green in (A)). Solid curve (Red, accessory): spectrum of iodopsin corrected by the transmittance curve of oil droplet-corresponding area in inner segment of accessory member (dotted curve in (A)). (Modified from Okano et al. [3].)

visual cell was determined [22]. Since the incident light to the retina comes into a visual cell from the synaptic side, the oil droplets act like colored filters.

Fig. 3A shows transmittance curves of oil droplets recorded by Bowmaker and Knowles [23]. In Fig. 3B action spectra of the double cone are shown, where the spectra of iodopsin (chicken red) are corrected with the transmittance curve of green oil droplets in the principal member (Green in Fig. 3A) for comparing with the chicken photopic sensitivity curve (closed circles in Fig. 3B). In case of an accessory member, which has no oil droplet, the spectrum was corrected with the transmittance curve of an area corresponding to the location of oil droplet in the inner segment (accessory member of double cone in Fig. 3A). Both the corrected spectra (solid curves in Fig. 3B) are very close to chicken photopic sensitivity curve which were electro-physiologically measured by Wortel et al. [24]. Therefore, one may suppose that the double cone would be responsible for light intensity discrimination in daylight vision rather than the color discrimination. Thus the single cones should be responsible for color vision.

The spectra of chicken red, green and blue are shown by dotted curves in Fig. 3C. They were corrected by transmittance curves of red, yellow and clear oil droplets (Fig. 3A), respectively. The spectrum of chicken violet was not corrected, because the blue oil droplet has no absorbance in the visible range. After the correction (solid curves in Fig. 3C), not only each peak of the spectra shifted to longer wavelength, but also the overlaps of two spectra, that is, chicken red and blue and chicken green and violet, were remarkably reduced. Thus, in the retina every monochromatic light below 600 nm with permissible exceptions can be absorbed by only two types of cone pigments, though red light above 600 nm can be absorbed by only chicken red.

In an animal having a dichromatic system like a gecko, though the gecko's color vision is not proved yet, every color must be absorbed by gecko green and blue. The same is true in monochromatic light. Thus the animal would specify a ratio between absorptions by the two pigments as a corresponding color, in other words, wavelength. Then one can suppose that the animal would recognize each color as corresponding monochromatic light. Therefore every color including monochromatic light can be positioned by the ratio on a straight line. In case of a gecko, for

instance, 530 nm light can be specified by a ratio between relative absorbances at 530 nm on the spectra of gecko green and blue (Fig. 1C), so that one can plot it on the straight line with Green and Blue at long and short wavelength ends, respectively (color line) (Fig. 4A). Namely the relative absorbance gecko green or blue at 530 nm in Fig. 1C corresponds to the distance from a point at 530 nm on the color line to Blue or Green end, respectively.

In a trichromatic system like human, all the colors are usually expressed by a regular triangle with human red, green and blue sensitive pigments (Red, Green and Blue, respectively) as the vertexes (color triangle) [13], on the assumption that white light would be absorbed equally by the three cone pigments. Then, white light can be positioned at the center of the color triangle, so that lengths of the three perpendiculars are identical with each other (R, G and B in the color triangle). Any light from 650 to 550 nm, for example, 600 nm light can be discriminated by an own ratio between absorptions by human red and by human green from other colors and monochromatic lights. Then, the absorption (relative absorbance in Fig. 1B) by human red or green corresponds to the length of the perpendicular to the opposite side of the Red or Green vertex (Fig. 4B), respectively. Thus, every wavelength from 700 to 380 nm can be positioned like a closed circle curve in the color triangle (wavelength curve). Below 500 nm, the wavelength curve enters into the triangle, because the wavelength can be specified by a ratio of absorption among the three cone pigments.

In a tetrachromatic system like chicken, all the colors can be expressed by a tetrahedron [13] with chicken red, green, blue and violet (Fig. 4C). When no correction by the oil droplet was made, all the light below 550 nm must be absorbed by the three cone pigments, so that the wavelength curve is positioned on the triangle having chicken red, green and blue as the vertexes of the color tetrahedron (Red, Green and Blue, respectively). Every light below 500 nm must be absorbed by the four pigments. Thus the wavelength curve enters into the tetrahedron.

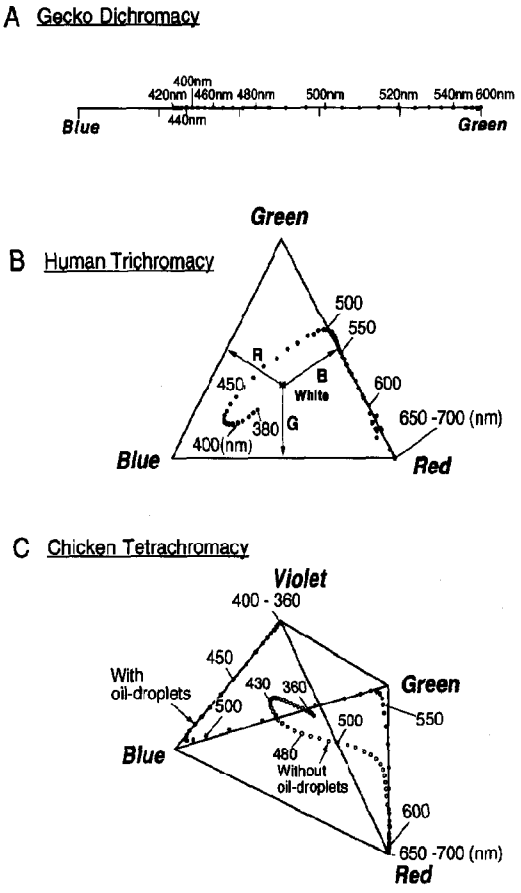

Fig. 4. Color diagrams (see text). (Modified from Okano et al. [3].) (A) A color line for gecko dichromacy constructed by calculating the ratio between relative absorbances at a wavelength of spectra of gecko green and blue (Fig. 1C). Every monochromatic light (closed circles) is plotted at 5 nm intervals on the straight line. (B) A color triangle for human trichromacy. The wavelength curve (closed circles) denoting locus of every monochromatic light (5 nm intervals) is drawn in the triangle by plotting the ratio among relative absorbances at a wavelength of spectra of human red, green and blue (Fig. 1B). White light is placed at the center of the triangle, because lengths of the three perpendicular (G, B, R) are identical with each other. (C) A color tetrahedron for chicken tetrachromacy. A wavelength curve (open circles) denoting locus of every monochromatic light (5 nm intervals) is drawn in the tetrahedron by plotting the ratio among relative absorbances at a wavelength of spectra of chicken red, green, blue and violet (Fig. 1A). Another wavelength curve calculated from the spectra of cone pigments corrected by the transmittance curves of the oil droplets (Fig. 3C) is shown by closed circles. It should be noted that the corrected one lies on the edge of the tetrahedron.

When the corrections with transmittance curves of the oil droplets were made, almost every monochromatic light from 600 to 400 nm must be absorbed by two cone pigments, so that the wavelength curve (closed circles in Fig. 4C) is located on the edges of the tetrahedron. An interval between two circles on the corrected wavelength curve, for example, between 550 and 450 nm is much longer than the corresponding one of the non-corrected wavelength curve (open circles in the tetrahedron). This would result in an increase of resolution of wavelength discrimination, although the elongation of the wavelength curve by the correction cannot be detected at the wavelength range near the corners of the tetrahedron.

Chicken has four types of cone pigments, the absorption maxima of which are regularly distributed on the wavelength scale, more than those of human three cone pigments. In addition, the retina has oil droplets. Comparing human, chicken has a good cone system. Thus one may imagine that chicken would enjoy richer color life than human does.

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