The diversity of cones in the retina of vertebrates: a review

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Abstract
Introduction
The most miraculous structure of the eye is the retina, a receptive layer containing a lining of closely packed visual cells/photoreceptors. It is well known that the retina of the vertebrates contains two types of visual cells: the rods and cones. The rods are specialised for dim vision while the cones are for bright and colour vision. The cones can be further subdivided into at least three subcategories: the single cone, twin cone and the double cone. The ordinary single cone usually exists alone, while the twin cone and the double cone represent two cones adhering to each other. The two components of the twin cone are identical in morphology. By contrast, the double cone consists of a tall and slender component, which is termed as the chief cone, and a rounder and shorter component, which is known as the accessory cone. The single cone is the most common type that can be found in most species of vertebrates, while others are less frequently seen, but still appear in many species up the vertebrate scale from fish to mammals. The aim is to introduce the variety of cone cells in the retinae of some selected species of vertebrates and discuss their structures with regard to the possible functions.

Conclusion
This critical review illustrates the diversity of cones in selected retinæ of animals. The cones and their internal components are phylogenetically old. Yet, the co-ordination of structure and function are unknown.

Introduction
The retina is a receptive layer containing a lining of closely packed visual cells/photoreceptors. This critical review discusses the variety of cones in the retina of vertebrate species.

Retinal layers
The receptive layer of the eyes in vertebrate is the visual cell layer of the retina. The cell bodies of the visual cells are concentrated into two to more layers termed as the outer nuclear layer. The thickness of the outer nuclear layer, corresponding to the number of the visual cells, thus varies in different vertebrates. For example, the primitive sturgeon, which is an archaic fish, has a thin outer nuclear layer and a thin inner nuclear layer, indicating a very few number of retinal cells in this species (Figure 1). This is very much in contrast to the retina of some other vertebrates such as the salmon and the chicken where the outer nuclear layer contains more cone and rod cell bodies and the inner nuclear layer is also relatively thick (Figure 2). In the avian, however, a very clear outer plexiform layer separating the outer and inner nuclear layers can be seen as well (Figure 3).

Different types of visual cells
It is well known that the retina contains two categories of visual cells: the rods and the cones. The rods are specialised for dim vision while the cones are for bright and colour vision. Amongst the latter category, the cones can be separated into at least three subcategories,

Figure 1: The sturgeon retina with a thin outer (O) nuclear and an inner (I) nuclear layer of very few cells. (vascular endothelial growth factor immunohistochemistry, ×400).

Figure 2: The chicken retina with inner nuclear layer containing a lot more cells compared with Figure 1a (arrow) (haematoxylin and eosin, ×200).

Figure 3: The avian retina as exemplified in the duck has a clear outer plexiform layer (OP) separating the outer nuclear (O) and inner nuclear layer (I) (haematoxylin and eosin, ×200).

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and these are the single cone, twin cone and the double cone. The single cone usually exists alone (Figure 4) while the twin cone represents two identical cones adhering to each other in appearance (Figure 5). The double cone also has two components ‘glued’ to each other, but these components are not similar in structure. Usually, there is a taller and slender component which is termed as the chief cone while another rounder and sometimes shorter component termed as the accessory cone (Figures 6–9). In general, all three types of cones contain outer segments of stacked membranes with transmembrane photopigments and inner segments with cell bodies containing mitochondria, rough endoplasmic reticulum and Golgi apparatus (Figure 10). In some species of fish to birds, the inner segments contain oil droplets (Figures 5, 7 and 8), which are β-carotenoids in nature in the distal tip just below the cilia, where the inner segments connect with the outer segments of the visual cells.

**Oil droplets and glycogen bodies in cone cells**

It was widely accepted that birds, reptiles all the way to the primitive fish have inner segments with oil droplets. In the study with different fishes, we had recorded the presence of oil droplets in the cones of a phylogenetically old fish, the sturgeon, though most of these droplets were colourless in this species (Figure 5), unlike those in the chicken (Figure 11). It is not entirely clear whether these droplets inside the inner segments exist as a light filter, as storage of energy or have other purposes. The fact that these oil droplets could have many colours led to a hypothesis that they might be...
related to spectral sensitivity. The yellowish oil droplets in the retina of the fish had been related with reduction of chromatic aberration, reduction of glare and dazzle, and the improvement of contrast vision. Most of the birds, if not all, have oil droplets in their inner segments of the retina. These include two active predators, which are the eagles and the owls. These birds display sharp acuity of vision. In addition, oil droplets are also seen in birds that are not good at flying such as the chicken and the duck. These ‘land’ birds, though are not aggressive predators as the eagles and owls, do need good vision to pick up small bits of food (e.g. worms). Our group performed an experiment years ago to observe retinal changes in the birds after occlusion of one eye. After a month, many of the oil droplets were gone. It was, however, not certain whether there was a disintegration of the oil droplets alone or a death of the cones with droplets or both. It was also doubtful whether the oil droplets were used as an energy source in the eyes of the birds as birds had a pectin oculi in their eyes, which were highly vascularised and could be an energy source.

Another peculiar structure inside the inner segments is the presence of glycogen bodies in the accessory cones. These glycogen bodies are present as significant structure in the myoid portion of the inner segments. Developmental studies on the chicken retina indicated an initial accumulation of rough endoplasmic reticulum in paraboloid regions along the long axis in the myoid region of the inner segment of the accessory cones. As the retina developed, the Golgi apparatus became active in this region and by around 6–7 days before hatching, glycogen began to form in between the rough endoplasmic reticulum. Collections of glycogen increased before hatching and these glycogen bodies with the rough endoplasmic reticulum formed round structures termed as paraboloid. Paraboloids (Figure 12) persisted in the adult chicken retina and those of other birds. The function of the paraboloid is not clarified at this stage. One of the possibilities is that this represents high energy storage and perhaps equipped for the high energy demand of the avians, particularly for those active predatory species. One support for this was from the previous work of our laboratory in which we occluded the eyes of the chicken for 1 month, the paraboloid began to lose their glycogen indicated by the less Periodic Acid Schiff-positive staining. In addition, most species in the lower vertebrate scale do have an equal and equivalent structure in their cones. This is exemplified by the sturgeon, which has a collection of glycogen in their accessory cones although this collection was often semilunar in shape (Figure 7). Furthermore, the appearance of glycogen was often documented in degenerating retinae of the fish, which obviously was not for providing energy in this already degenerating structure.

**Diversity of cone cells**

In the albino rat, it was generally agreed that the great majority of the retinal visual cells were rods, and cones were extremely rare. In the deep sea species, as exemplified by the sharks, cones were present in the retina but in very small quantities (Figure 13), while in the amphibians, double cones persisted (Figure 14). However, one must be aware that the sharks do not use vision to catch prey. In the fresh water fishes, such as the sturgeon, twin cones are demonstrated in the retina (Figure 15). In these animals, the ratio of twin cone to single cone was about 1 to 10

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**Figure 10:** Inner segment of accessory cones contain not only glycogen and endoplasmic reticulum, but also Golgi apparatus (A) after fracture of the surface (scanning electron microscopy, ×1500).

**Figure 11:** The retina of a chicken showing red and yellow oil droplets (whole mount, ×40).

**Figure 12:** Paraboloid (P) in the accessory cones of the chicken retina. They contain glycogen and thus are PAS positive. Normally, they are oval in shape in the chicken retina (periodic acid-Schiff, ×400).

**Figure 13:** The retina of the shark showing a lot of rods (R) but few cones (C) (periodic acid-Schiff, ×400).
Review

Competing interests: none declared. Conflict of interests: none declared.

All authors contributed to the conception, design, writing, and supervision of the manuscript, as well as read and approved the final manuscript.

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and the double cone to single cone ratio was about 3 to 4, with the rest as rods. For detailed comparison between animal groups, please refer to our comparative atlas published in 2012 (ref. 6). Since the active predators, which dwell in shallow waters such as the crocodiles, possess a fair amount of twin cones in the retina, their functional role in the deep sea does not appear to be supported. In these predators, the fact that the number of double cones and single cones were close to each other suggested that both were important in vision (e.g. colour vision) associated with predation activities.

Electrically, the double cones and twin cones possess diverse properties. Injection of fluorescent dye into one component of the twin cone reported a lack of dye coupling into the other cell of the twin cone but electrical coupling was evident while double cones displayed dye coupling between the two components7. Electrical coupling was observed in the gap junction of many cones in the mammalian retina8 and in between red and green cones in the primates9. In our morphological studies, it was clear that components of double cones adhered tightly in the proximal region above the outer limiting membrane but not so tightly in the more distal areas (Figure 16). Furthermore, both components of twin cones adhered tightly all the way (Figure 17).

The cones in the retina are usually present in specific pattern. It is already well known that cones are concentrated more in the central retina than the periphery. This statement is not flawless. Near the ora serrata of the extreme periphery of the retinae, cones are still present in fair amounts in most of the species studied in the laboratory (Figures 18). It is possible that in the lower vertebrates, such as the fish, they may be used to sense the reflection of the colour in the water to avoid the approaching danger. However, cones (usually single cone) near the ora serrata were also observed in primates, e.g. monkey (Figure 19). In this case, they might be useful in the search of food,

Figure 14: A double cone (encircled) and many rods in the frog retina (periodic acid-Schiff, ×400).

Figure 15: The retina of a crocodile showing two slender cones adhering together as twin cones (arrow) (scanning electron microscopy, ×3500).

Figure 16: Retina of the Siamese fighting fish showing pairs of double cones (arrow). C stands for slender chief cones and A for accessory cones (scanning electron microscopy, ×2000).

Figure 17: A possible type of double cone in the monkey retina with a slender chief cone (C) and a slightly round accessory cone (A). On the other hand, a pair of twin cones (arrow) appeared as slender cones adhering together all the way from the inner segments to the outer segments (scanning electron microscopy, ×4000).

Figure 18: The retina of the turtle showing single cone (S) in the peripheral retina near the ora serrata and an oil droplet (O) in the accessory cone of the double cone, also in the peripheral retina (haematoxylin and eosin, ×400).

Figure 19: Another type of double cone in the retina of the monkey. In this case, the chief cell (C) is slender but not taller than the accessory cone (A). The accessory cone still has a rounder shape (scanning electron microscopy, ×4000).
neighbouring cells. Further studies from inner segments contacting which many cones had appendage we found in the chicken retina, in FĔė ĈĎęĆęĎĔē ĕĚėĕĔĘĊĘ:

while 10%–12% contained S opsin10, of the cones contained L/M opsin, In the monkey retina, using this different types of opsins in the cones. by immunostudies targeting on the morphologies could be visualised any concrete answer, the cones' different types of cones is still beyond tional importance which remains to

rods (Figure 20). This may have func-

surrounded by a mosaic of many (Figures 9 and 17). The double cones mostly seen in the central retina (cones were less frequent and were mostly seen in the central retina. Twin cones were less frequent and were TWIN CONES WERE USUALLY SURROUNDED BY A MOSAIC OF MANY RODS (FIGURE 20). THIS MAY HAVE FUNCTIONAL IMPORTANCE WHICH REMAINS TO BE ELUCIDATED.

Although the morphology of the different types of cones is still beyond any concrete answer, the cones’ morphologies could be visualised by immunostudies targeting on the different types of opsins in the cones. In the monkey retina, using this approach, it was clear that 88%–90% of the cones contained L/M opsin, while 10%–12% contained S opsin10, and both S and L/M opsin appeared in the fovea during foetal life while S opsin expression was more peripherally situated than L/M opsin10. It was interesting that in this work, cones with S opsin had appendages that might reach neighbouring cones10, which was similar to what we found in the chicken retina, in which many cones had appendage from inner segments contacting neighbouring cells6. Further studies on cone morphology in the human retina described the first appearance of S opsin protein in the fovea at around 11 foetal weeks while L/M opsin appeared in the fovea 4 weeks later, as well, S opsin mRNA and protein were detected much farther into the peripheral retina than L/M opsin11. S cones covered much of the retina by 19th foetal week while L/M cones reached the edge of the retina by 34–37 weeks of gestation and near term11. We had in fact a collection of scanning electron microscope (SEM) images showing morphological evident of cones in the human retina after 20 weeks of gestation6, suggesting the time when the cones had assumed their adult structural configuration.

The exact function of the different cones remains to be elucidated. It is tempting to assume that there are different types of physiological active spectral cones which respond to different wavelength, the S, M and L spectral cone cells, as in the human retina. The L cone cells are sensitive to long wavelength with a red colour peak. The medium or M cone cells respond to medium wavelength and peak at a green peak, while the S cone cells receive short wavelength and peak at the blue region. There was much overlap between the M and the L spectral cone cells. But how does this theory relate to the cones of different morphology? Or if they correspond at all remains a question to many researchers because there is no acceptable answer. It has been suggested that different coloured oil droplets were present in the visual cell of the vertebrate all the way to marsupials12,13 and they play a role in spectral sensitivity of various cones; for example, four types of single cones in birds14-17. In the pikeperch, it had been suggested that the chief cone was active for red colour absorption while the accessory cone was active for green colour absorption and the maximum activity of the twin cones was at 605 nm, also at the red spectrum19. Does that mean the yellow spectrum is covered by the single cones or not? What is the spectral difference between twin cones and double cones? It had been documented that many elasmobranch had pure cone retina while sharks and rays started to acquire both rods and cones7. Most teleosts displayed single and twin cones while the latter were mainly located in the temporal retina7. In some deep sea fish, cones were absent7, whereas in other studies, twin cones were observed to increase with increasing depth in the sea and were assumed to be related to the depth of their niche20.

Discussion

The authors have referenced some of their own studies in this review. The protocols of all our highlighted studies were approved by the relevant ethical committees related to our institution in which it was performed. Animal care was in accordance with the institution guidelines.

Degeneration and plasticity of cone cells

Generally, visual cells were prone to degenerate on injury in vertebrates21. Degeneration of the retina in aged animals was also demonstrated by the changes in the number of optic nerve during aging20. Interestingly, in the degenerating retina, the cone cells usually survive to the last. In the fish, degeneration in the retina would first

Figure 20: Observe the accessory cones (A) surrounded by other visual cells, particularly rods. Monkey retina (scanning electron microscopy, ×1000).

Figure 21: Surviving cone cells (arrow) were the last components of the visual cells in the retina of the crocodile (haematoxylin and eosin, ×400).

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attack the rods and leave the cones to the last to degenerate, as in the mutant goldfish and the goldfish that had optic nerve sectioned.14,18 This was same as in other animals, e.g. the crocodile (Figure 21). This illustrated that the cone cells were particularly tough in survival. In the mutant goldfish, for example, the cells that were still surviving in the retina upon degeneration were the cones which were still there when all other cells and layers had perished.19 Moreover, some visual cells would survive even if they were detached from the brain or the secondary or tertiary neurons. This notion had been proved in the developing rat retina after sectioning of the optic nerve, the retina still developed into the adult form20, and in the degenerating human foetal brain where development and maturation of the retina had not been prohibited21. In other studies, detachment of the cat retina would promptly cause degeneration of cone cells. For instance, S cone opsin decreased to 42% of the normal 3 days after detachment and M cone opsin decreased to 4% but returned to normal after reattachment.22 Does this mean that reattachments survive degenerating visual cells or there is regeneration of visual cells? Classically, regeneration of visual cells has not been reported in higher species. However, in some of our studies, we had observed small and seemingly immature visual cells (Figure 8). To this date, although many of the information on cones have been added to the literature, a comprehensive correlation on the different morphological types of cones with physiology, biochemistry and pathology are still lacking. Studies on these lines are still fruitful.

Conclusion
This critical review illustrates the diversity of cones in the retinae of some selected animals. The cones and their internal components, e.g. oil droplets and glycogen bodies, are phylogenetically old and could be dated back from the primitive fish such as the sturgeon. Yet, the coordination of the structure and function are elusive. We call to see more research on this topic to help advance our thinking and help clarify any previous controversies.

References