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Morphology of Retinas and Lenses in the Fish of the Genus *Zacco* (Cypriniformes, Cyprinidae): Possible Relationship with Prey and Habitat

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Vertebrates with different habitats have different proportions of visual cells, with the rod cells responding to scotopic vision and the cone cells responding to photopic and color vision in their retinas. The present work studied whether the kinds and arrangement patterns of the cone cells and interlocking morphology of the lens were related to the kind of preys and habitats in the genus *Zacco*. The retinas were observed by a light microscopy using H-E staining method and the interlocking formula of the lens fibers were investigated by a scanning electron microscopy. The interlocking formula of the lens fibers of *Z. temmincki* is an "anchor and socket" connection, and that of *Z. platypus* is a "ball and socket" connection. The cone cells of *Z. platypus* and *Z. temmincki* constituted compacted mosaic patterns of row type. Away from the center, the double and single cone cells gradually increased in diameter. *Zacco temmincki* had identical double cone cells and *Z. platypus* had non-identical double and single cone cells. The eyes of *Z. temmincki* feeding on a moving aquatic insects in relative limpid water and swift current of mid and upper stream have better resolution than that of *Z. platypus* feeding on mainly adhesive algae and some aquatic insects in slightly turbid water of mid stream.

The vertebrate retina has been subjected to extensive studies, because of its accessibility, simple layered structure, well defined cell types and patterns of connection, and because of interest in understanding the retina itself and in using the retina as a model system for the central nervous system (Han and Walter, 1997). Vision plays a critical role in the early life stages of most teleost fish, as the timing of eye development and establishment of functional vision is essential for perception of food (Dabrowski, 1982) and avoidance of predators (Fuiman and Magurran, 1994). Fish with different habitats and food have different proportions of visual cells in their retinas, and some nocturnal species lack cone cells entirely (Locket, 1977; Nicol, 1989).

These facts are among those that led Schultze (1866) to formulate the duplicity theory. In accordance with this theory, it is now generally considered that scotopic vision is mediated by rods and photopic vision by cones (Ole, 1984; Nicol, 1989). In teleosts, most cones are single or double cells, but triple and quadruple cones occur occasionally in some species (Lyall, 1957; Engström, 1958, 1960, 1963; Nicol, 1989; van der Meer, 1992; Nishiwaki et al., 1997; Cook and Chalupa, 2000; Carl, 2001; Lim et al., 2002; Mochizuki, 2002). And double cones are divided into equal double cones (twin cones) and unequal double cones by the cell size only. Equal double cones are subdivided into identical double cones and non-identical double cones by visual pigments contained (Lyall, 1957; Nicol, 1989; van der Meer, 1992). Such cones are distributed irregularly in some species. It is usual, however, to find single and double cones being arranged in specific patterns (Lyall, 1957; Van der Meer, 1992; Shusaku et al., 1999). Most retinas comprise an outer and inner retinas. The former is made up of an epithelial layer, visual cell layer, and external limiting membrane. The latter is a nervous tissue, comprises 6 or 7 layers (Ali and Anctil, 1976; Nicol, 1989).

In the genus *Zacco*, dark chub, *Z. temmincki* and pale chub, *Z. platypus* are abundant in Korea, and distributed in Japan and China (Kim, 1997). *Zacco temmincki* inhabits swift current and mainly preys on the aquatic insects. *Zacco platypus* on the other hand inhabits swift and gentle currents, eats mainly the adhered algae or

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some aquatic insects (Kim, 1997). The purpose of the present work is to comparatively study the structure and interlocking morphology of the lens fiber, the kinds of cells, and the cone mosaic patterns of the retinas by a light microscopy and a scanning electron microscopy in *Z. temmincki* and *Z. platypus*. From the result, we discuss distinction of food and habitats in relation to structural differences of the lens and retina.

Materials and Methods

Animals

In this study, 11 specimens of *Z. temmincki* (103.7~113.0 mm, TL) and 10 of *Z. platypus* (125.9~137.0 mm, TL) were collected at Jeonju stream, Jeonju, Jeollabuk-do, Korea in June and July, 2002.

Histology

Whole eyes were kept in 10% formaldehyde for 1 h and the horizontal and vertical diameters of the eyeball and pupil were measured by microtome (Mitutoyo). The eyeballs were hemisected, the cornea, iris and lens were removed, and the posterior eyecup were cut into quadrants for 1-2 d of further fixation. After washing, the quadrants of eyecup were divided into the upper and lower parts, and each parts were subdivided into central and three peripheral sections. Eight fragments (2×2 mm) for transverse section (Fig. 1D-K) and three fragments (3×4 mm) for vertical section (Fig. 1A, B, C) were cut out mainly from the right eye. The samples were subjected to successive course of dehydration, cleaning, paraffin infilteration and embedding. For histological examination, the retinal fragments were dehydrated through a standard ethanol series to 100%, cleaned in xylene and then embedded in wax (Paraplast, Oxford). For light microscopy, we deparaffinized 4 μm sections and stained them with Harris' hematoxylin with eosin. For visual observation, we examined sections of the retinas by light microscopy (Olympus BX 41) and Image plus pro 4.0 (Media cyber netics).

Scanning electron microscopy

Both sides of the lens were cut out to the degree of one-forth and one-third (Nicol, 1989) for scanning electron microscopy (SEM). The samples were prefixed in cold 2.5% glutaraldehyde for 2 h. After rinsing with 0.1M cacodylic acid-sodium salt trihydrate (CB) (2 times), the samples were postfixed in 1% osmium tetroxide for 1 h and then rinsed with 0.1M CB (2 times). The samples were then dehydrated in a graded ethanol series. After three changes (20 min each) of 100% ethanol, the samples were subjected to critical-point dry in isomyl acetate and hexamethyldisilazane (HMDS) series. After one change (10 min each) of pure HMDS, the samples were mounted on stubs, sputtered with gold, and immediately observed with SEM (Akashi SR-50).

Results

Morphology of lens fiber

The lens in *Z. temmincki* and *Z. platypus* are globular in shape, and originally occupied a central position within the pupil.

The interlocking interdigitations of the lens fiber were evident at the edges along the length. The interlocking morphology of the lens fibers was an "anchor and socket" connection, and the width of the lens fibers was 6.5 μm in *Z. temmincki* (Fig. 2A). While the interlocking

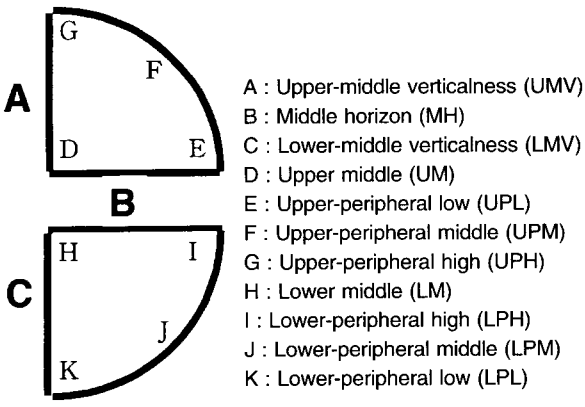

Fig. 1. Idealized position of hemi-eyecup for the transverse and vertical sections.

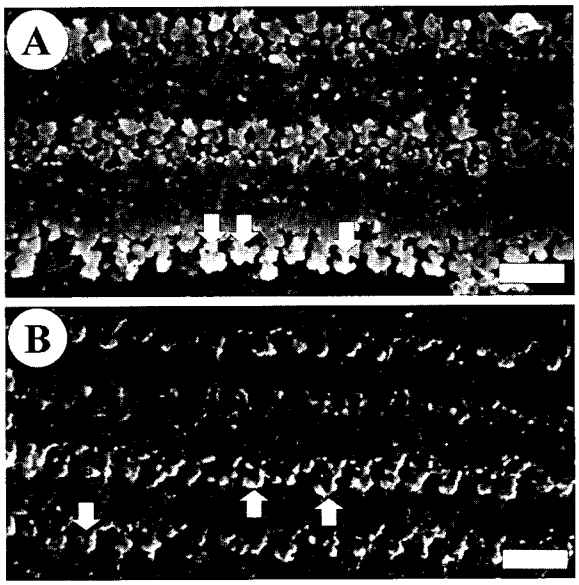

Fig. 2. Structure and interlocking morphology (arrows) of lens fibers of *Z. temmincki* (A), and *Z. platypus* (B) by SEM. Scale bar=5 μm.

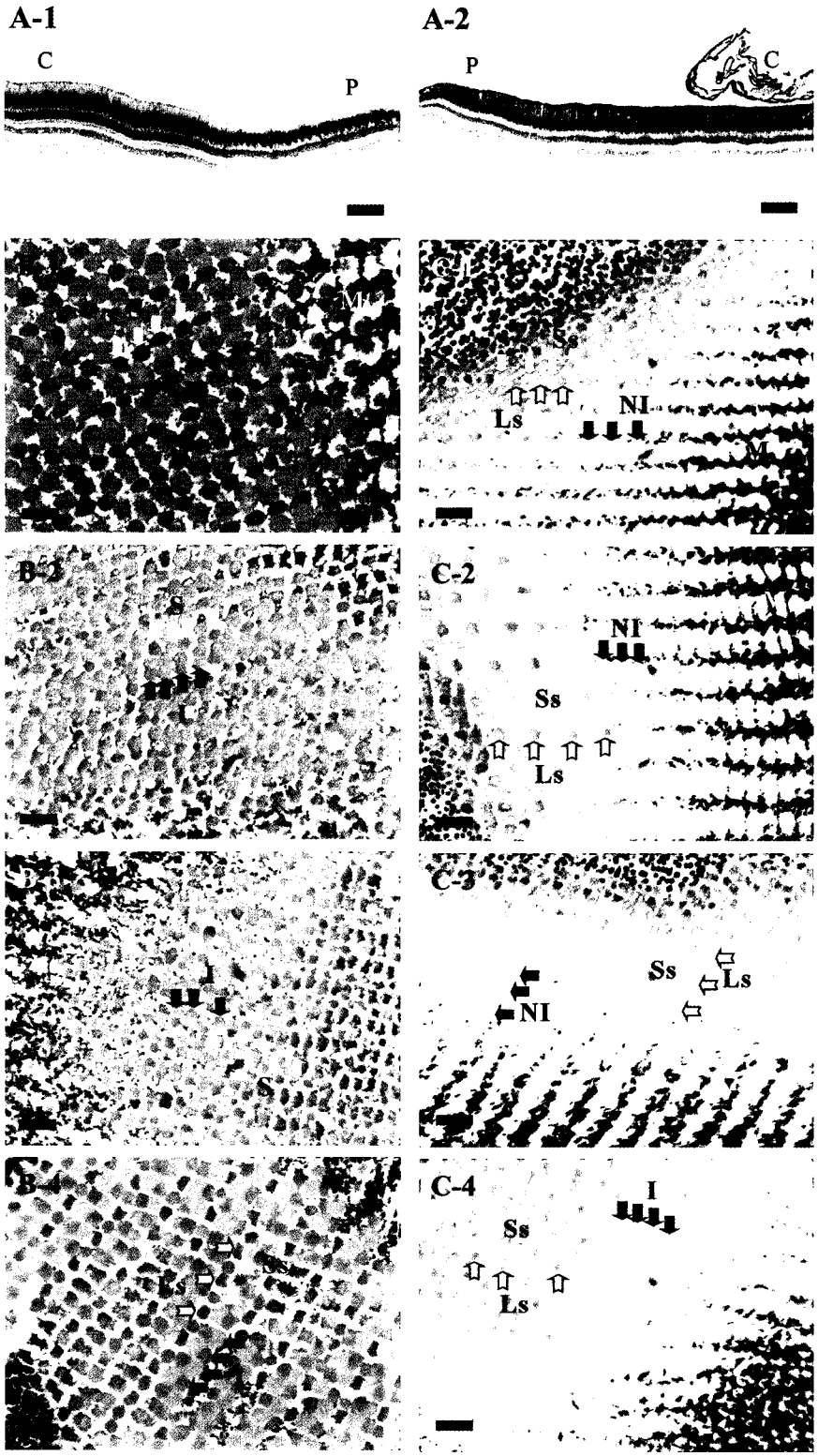

Fig. 3. Structure of retinal tissues in *Z. temmincki* and *Z. platypus*. A, Vertical section of retina in *Z. temmincki* (A-1) and *Z. platypus* (A-2). B, Transverse section of retina in *Z. temmincki*. C, Transverse section of retina in *Z. platypus* in the upper-peripheral high and lower-peripheral low (B, C-1), in the upper-peripheral middle and lower-peripheral middle (B, C-2), in upper-peripheral low and lower-peripheral high (B, C-3), in the upper middle and lower middle (B, C-4). C, central portion; I, identical double cone; Ls, long single cone; M, the region where pigment processes surrounding cone cells; NI, non-identical twin cone; P, peripheral portion; S, single cone; Ss, short single cone. Scale bars=60 μm (A) and 5 μm (B, C).

morphology of lens fibers was a "ball and socket" connection, the width of lens fibers was 4.8 μm in *Z. platypus* (Fig. 2B).

Retinal histology

Vertical sections of the retinas

The outer nuclear layers of *Z. temmincki* and *Z. platypus* were developed more than the inner nuclear layers of those. The processes of pigment epithelium were a light-adapted phase and thus the pigmented processes penetrated among the visual cells. In total, the retinal thickness was highest in the central base and decreased towards the margin in both species (Fig. 3A-1, 2).

Transverse sections of the retinas

The cone patterns were clearly found in transverse sections of the retina in *Z. temmincki* and *Z. platypus*. The cells were arranged so that double cone rows were running radially, two rows of single cone being separated by a row of double cones in both species.

In *Z. temmincki,* the sections of the upper-peripheral high (UPH) and lower-peripheral low (LPL) consisted of largely identical double cones with about the same pigments and single cones (Fig. 3B-1). The 2nd type of the arrangements was found in the sections of the upper-peripheral middle (UPM) and lower-peripheral middle (LPM). The sizes of these identical double and single cones were smaller than those of cells in the UPH and LPL (Fig. 3B-2). The 3rd type was in the upper-peripheral low (UPL) and lower-peripheral high (LPH). The identical double cones with various pigments and single cones were distributed in the UPL and LPH (Fig. 3B-3). The last pattern, the 4th type was located in the upper and lower middle (UM and LM). Here we found that the long single cones (Ls) and short single cones (Ss) were alternately situated between the clear identical double cones (Fig. 3B-4).

In *Z. platypus*, the kinds and arrangement pattern of cone cells were different from those of *Z. temmincki*. The four kinds of cone cells, i.e., short and long single cones, identical and non-identical double cones, were found at the eight sections. In *Z. platypus,* the arrangements of retinal cone cells were largely grouped into two types (Fig. 3C-1, 2, 3, 4). The 1st type was found in all of the parts except at the UM and LM. The short and long single cone cells were alternately situated between the non-identical double cone cells (Fig. 3C-1, 2, 3). The size of non-identical double cone cells was the largest at the UPH and LPL, became smaller toward the UPL and LPH (Fig. 3C-1, 2, 3). The 2nd arrangement type of cone cells was found in the parts of the UM and LM (Fig. 3C-4). In contrast to other parts, we found identical, instead of non-identical, double cones. Similar to other parts, short and long single cone cells alternated, and a row of identical double cone cells was situated between two rows of single cone cells (Fig. 3C-4).

The eyeball and pupil of *Z. temmincki* and *Z. platypus* were globular. The average sizes of eyeball and pupil in *Z. temmincki* were 7.5±0.44 mm and 3.6±0.44 mm (mean±SD), respectively. The average sizes of those in *Z. platypus* were 7.0±0.42 mm and 3.3±0.43 mm (mean±SD), respectively.

Discussion

The present study investigated the kind, shape, arrangement, and distribution of cone cells in relation to the positions of fish retina and also inquired into the shape and interlocking formula of lens fibers in *Z. temmincki* and *Z. platypus* in the genus *Zacco*. Several different types are found to be related with their adapted phases to food and habitats of *Z. temmincki* and *Z. platypus*.

The cone patterns of the two species in the genus *Zacco* appeared in a row pattern. But *Serrasalmus marginatus* and *Leporinus fasciatus* belonging to the same as the Superfamily Cyprinoidei have different square patterns (Ali and Anctil, 1976).

Good resolution is demanded on the high density of visual cells (Levine and MacNichol, 1979; Nicol, 1989). As in the case of *Z. platypus*, the sizes of single and double cone cells are generally larger than those of *Z. temmincki*. Thus, densities of the double and single cone cells appeared high in *Z. temmincki*. In general, middle and long-wave sensitivity are subserved by the double cone, while short-wave sensitivity is achieved by the single cone (Harosi and Hashimoto, 1983; Bowmaker and Kunz, 1987). The absorption maxima of the visual pigments of the non-identical double cones appears to be markedly smaller than those of the identical double cones in Cyprinids (Levin and MacNicol, 1979; van der Meer, 1992). As the identical double cone cells of *Z. temmincki* are totally developed compared to only the UM and LM of *Z. platypus*, we found that the eyes of *Z. temmincki* have a better resolution for detecting a moving prey in relative limpid water and swift current. In case of *Z. platypus* living on adhesive algae in slightly turbid water and gentle current, the long and short single cone cells are totally developed. The size of the double cone cells are the largest at the UPH and LPL, and become smaller toward the UPL, LPH, UM and LM in two species. As the sections of the UM and LM have the highest densities of the cone cells, those sections maintain the best resolution in the eyeball, and *Z. temmincki* and *Z. platypus* catch a prey well situated in front of the eye and head. The total length of *Z. temmincki* is shorter than that of *Z. platypu*, but the average sizes of eyeball and pupil of *Z. temmincki* are larger than those of *Z. platypu*. As the eyeball of *Z.*

temmincki is developed larger than that of *Z. platypus,* the cone cells of *Z. temmincki* are abundant than those of *Z. platypus*. As the pupil of *Z. temmincki* is larger than that of *Z. platypus,* and more quantity of transmitted light are reached at retina, the eyes of *Z. temmincki* have a good resolution (Nicol, 1989). The tight interlocking formula of lens fibers is associated with the transparency of the lens (Kessel and Kardon, 1979). In *Z. temmincki*, lens fiber makes a tight interlocking of "anchor and socket" type and the width of lens fiber is about 1.5 times wider than that of *Z. platypus*. In *Z. platypus,* however, the lens fiber makes a tight interlocking of "ball and socket" type, and is similar to the interlocking pattern of *Hemibarbus longirostris* living on adhesive algae in a slightly turbid water and gentle current of the downstream (Lim et al., 2002).

Therefore, we conclude that the kinds, arrangements and development of the cone cells in retinas, the interlocking morphology and widths of lens fiber, and developments of eyeball and pupil are related to good resolution in fish.

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