species belong to different genera and families, but normally there is a high degree of correlation between range of host species and range of higher host taxa, and an index based solely on species is therefore fully satisfactory.

* Dedicated to Prof. J.F.A. Sprent on the occasion of his 65th birthday.

1 Supported by grants from the University of New England and the Australian Research Grants Committee. All those acknowledged in 'Diversity gradients of marine Monogenea in the Atlantic and Pacific Oceans', K. Rohde, Experientia (this issue) are once again gratefully acknowledged.

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Cone mosaics in a teleost retina: Changes during light and dark adaptation

Yvette W. Kunz[1]

Department of Zoology, University College, Dublin 4 (Ireland), 17 April 1980

Summary. The square mosaic pattern of retinal cones in the guppy, *Poecilia reticulata,* changes during dark adaptation into a row mosaic. The functional significance of this change is discussed.

In most teleosts, the visual cells show a mosaic-like arrangement, with cones the dominating elements and rods interspersed at random[2,3]. There are 2 standard types of mosaic: in the row mosaic the contact zones between the partners of double cones are linearly arranged; in the square mosaic, the double cones form a zig-zag pattern, with the contact zones of 2 adjoining double cones forming an angle of 60 or 90°. In both types of mosaics, single cones usually occur. They are spaced at equal distances, forming rows parallel to, or intersecting with, rows of double cones[4,5-7]. A change from row to square pattern occurs during the ontogeny of a few teleosts[8-10]. Row and square patterns are also found in the same retina, occurring each in a different region[11,12].

In the guppy, *Poecilia reticulata* P., the square mosaic extends over the whole bulbus, except for a narrow peripheral growth zone[13]. However, when retinal fragments, obtained by immersing the eyes in calcium-free Ringer solution, were examined, they invariably showed a row mosaic (results unpublished). It was therefore decided to establish whether the entire retina, on being subjected to Ca-free Ringer, reveals a row mosaic, and if so, to investigate the causes of this pattern change.

Materials and methods. Adult fish (eye diameter > 1.6 mm), kept under a 12-h day and night cycle, were used. Light and dark adapted retinae were obtained by microdissection in Ringer solution either with or without Ca. For light microscopical analysis, whole intact retinae were mounted on slides and viewed with a Nomarski differential interference microscope (Zeiss), with Polaroid camera attachment. For electron microscopy, retinae were fixed in glutaraldehyde, postfixed in osmium tetroxide and embedded in araldite[17]. Ultrathin sections were stained with uranylacetate and lead citrate and viewed with a Philips 201C electron microscope.

Results and discussion. Light microscopy of whole light adapted retinae, freed in Ca-free Ringer, revealed that the cones over the whole eye bulbus had assumed a row mosaic pattern (figure 1). The occurrence of a row mosaic, in place of the square mosaic, could be attributed to the incubation medium used. Ca-free Ringer is used for retinal preparations, because it facilitates the detachment of the sensory retina from the adjoining interdigitating pigment epithelium and the pigmented chorioid[14]. Similarly, the absence of calcium may loosen the contacts between the different types of cones, thereby causing the disintegration of the square mosaic. The square mosaic in the guppy consists of double cones and long single cones in close apposition, whereas the short single cones are relatively isolated. The ellipsoids of long single and double cones show subsurface cisterns along the contact zone. Subsurface membranes also characterize the contact zone between the double cone partners (figure 2A). More vitreally, double and long single cones gear with each other by so-called fins (villous expansions of the cell membrane in the myoid region)[15].

Retinae treated with Ca-containing Ringer gave varying results. With this method, laborious microdissection is needed to separate the pigment epithelial processes which

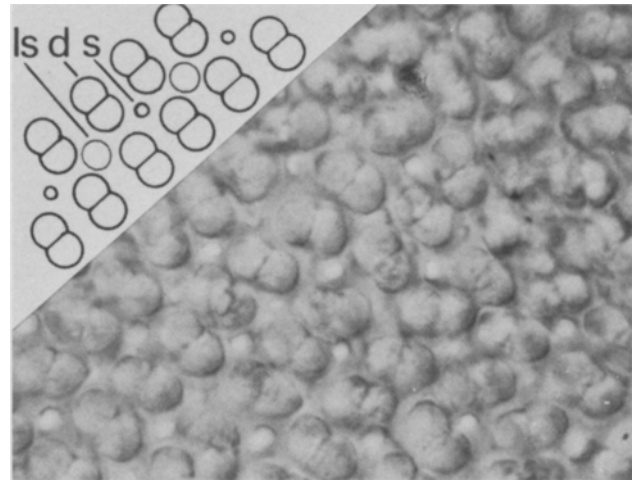


Fig. 1. Visual cell layer of the guppy, *Poecilia reticulata* (eye diameter > 1.6 mm). Photomicrograph taken by polaroid camera attached to Nomarski differential interference microscope, focussed to the level of double cone ellipsoids. Retina freed from pigment layers in calcium-free Ringer solution. Double cones are arranged in a row pattern, separated by rows of alternating long and short single cones. Abbreviations: d, double cone; ls, long single cone; s, short single cone. Magnification × 900.

interdigitate closely with the photoreceptors. In all the areas totally freed from the pigment epithelium, the row pattern was observed. However, in patches where only the basal layer of the pigment epithelium had been removed, leaving the processes in position, the square mosaic was evident. It seemed, therefore, that it is the presence of the pigment epithelium processes which is instrumental in preserving the square mosaic.

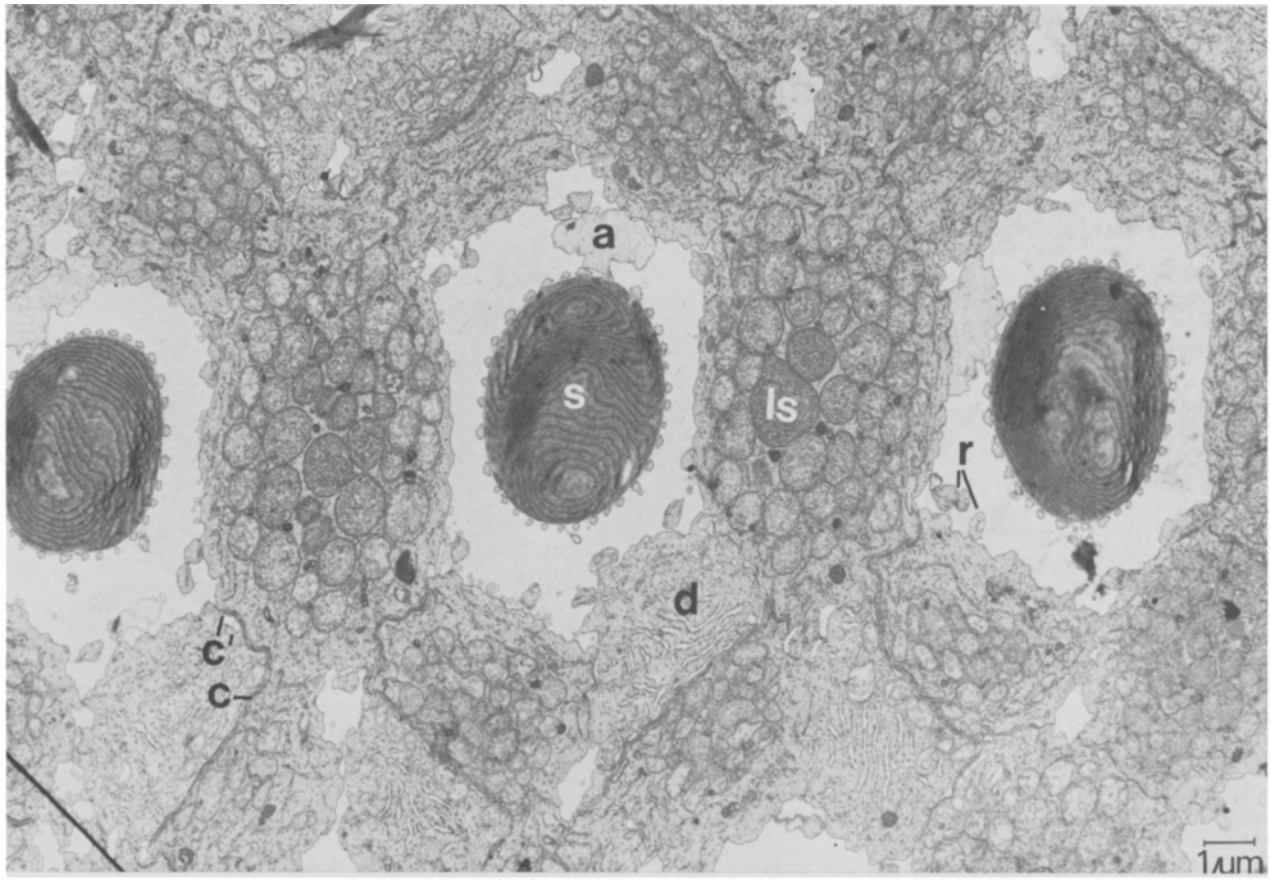
In the guppy, pigment epithelium processes are retracted under the physiological condition of dark adaptation[13]. If these processes are responsible for the maintenance of the square mosaic in the light adapted retina, the mosaic should change in the dark. Light microscopical observations showed that, indeed, in the dark the cones assume a row pattern over the whole retina. At the electron microscopical level, tangential sections revealed that the contact between double and long single cones was severed, and that the gaps were filled with the vitreally extended rods (figure 2B). Longitudinal sections showed that all the cones had moved sclerally, but at a different rate. While retaining the staggered arrangement, the ellipsoids of the long single cones no longer geared with those of the double cones (figure 3B). Moreover, no subsurface cisterns were observed in the periphery of the ellipsoids of either double or long single cones, now separated (figure 2B). However, serial sections would be necessary to establish whether, in the dark, the subsurface cisterns disappear along the entire former contact zone. Transverse sections taken more vitreally, revealed that in the nuclear region the square pattern, with the zig-zag arrangement of the double cones, had remained unaltered (figure 3B). It seems, therefore, that the scleral network of the Müller cell processes (external limiting membrane) provides mechanical stabilization at this level, both in the light and dark adapted state, while the fins and subsurface membranes stabilize the distal square pattern in the light (figure 3A). No other teleostean cell mosaic, to my knowledge, has been tested in the dark adapted state. It has been noted, however, that in the goldfish, which has a 'rudimentary' cell mosaic, there is 'wobble, drift and rotation' in the dark adapting cones[16].

The accessory outer segment[17], which connects the cone outer segments and the pigment epithelium, may be instrumental in orderly pattern changes. Tangential sections of light adapted eyes show that the arrangement of the accessory outer segment within the cone mosaic is regular. In longitudinal sections, it is seen to wrap around the cone outer segment. The experimental removal of the pigment epithelium (in Ca-containing Ringer) exerts a pull of the accessory outer segment; this separates double and long single cones resulting in a row mosaic.

Retinomotor responses were first noted over a century ago. Since then, the literature has been periodically reviewed and re-interpreted[18,19]. On the basis of my results, what is the significance of the photomechanical movements and the resulting changes of mosaic patterns in the guppy?

The pigment granules move vitreally in response to light and sclerally in the absence of light. At the beginning of the century it was decided that this migration is not due to the extension and retraction of cell processes but to the migration of granules in relatively fixed cells. This opinion seems to prevail to this day[18,19]. However, the electron micrographs of the guppy clearly show that the processes do contract and extend.

In the light adapted state, the extended pigment epithelium processes shield the rods from bright light. I suggest that, in addition, they provide a close anatomical contact with the rod outer segments to ensure the uptake and digestion of the discs, discarded after light stimulation[20]. During dark

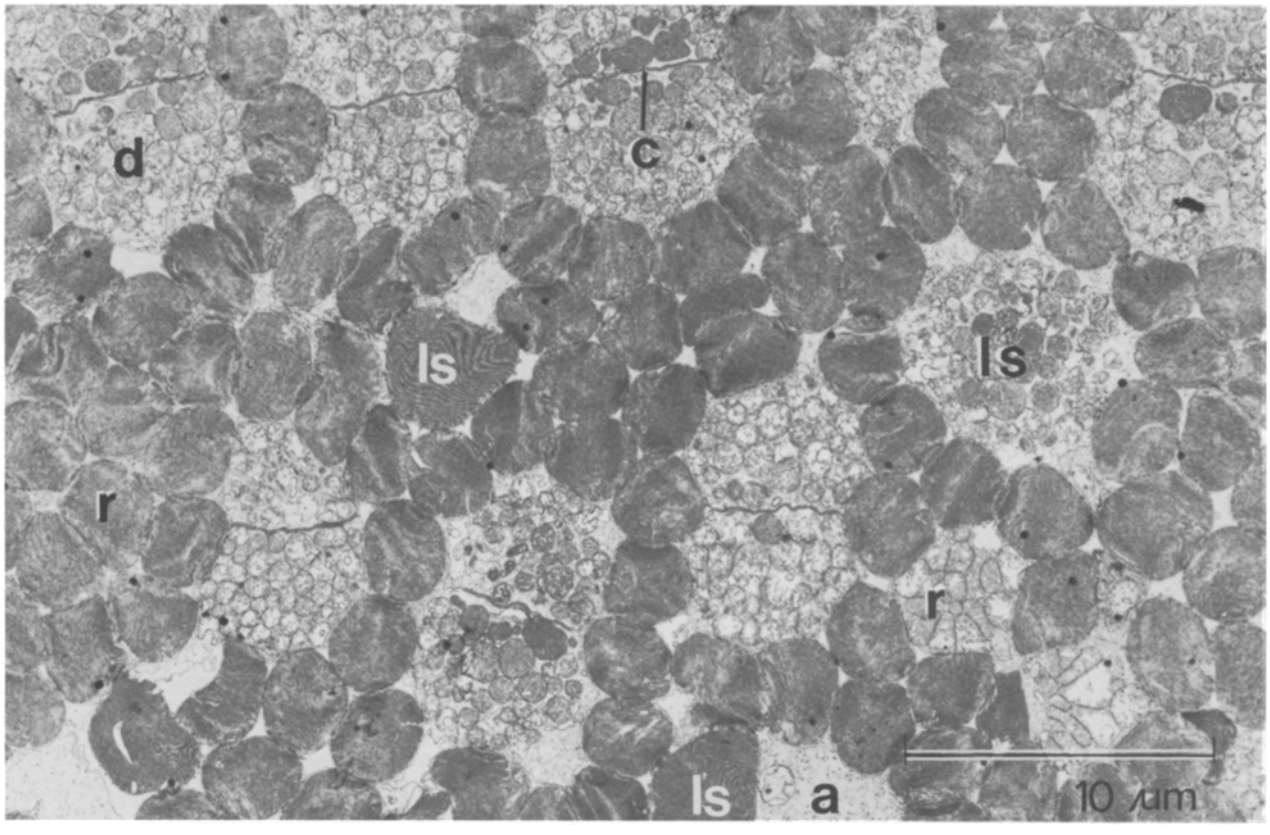


A

adaptation, the double and single cones extend, thereby retaining their close contact with the retracted pigment epithelium. This ensures the uptake of the cone outer segment discs, discarded only in the absence of light[21,15]. However, the outer segments of the vitreally positioned short single cones never become embedded in the pigment epithelium. The packages of discs, shed from the tips of these cones, may become engulfed by ameboid phagocytes, as observed in the goldfish[21]. The vitreal position of these cones, in dim light, may have an added functional significance. Their large ellipsoids, packed with mitochondria, and the low refractive index of the surrounding extracellular fluid, may act as optical guides to funnel light on the overlying rods (figure 3B)[22,23].

What then is the advantage, to the guppy, of a square mosaic, present in the light adapted state, over a row mosaic, as evidenced in dark adaptation? The square mosaic may enhance the perception of motion (obtaining of food, avoidance of predators, elaborate mating ceremonials). The square mosaic seems to be superior in that it allows the perception of movements from all directions, whereas row mosaics are restricted to perception from 2 directions[5-7,11]. Within the same eye, regions with row mosaics are for dim vision, those with square mosaics for acute vision[4]. It has been shown recently that within the same species, a change from square to row mosaic, during ontogenesis, is associated with the permanent migration of the fish from the surface (bright light) to deep waters (dim light)[24].

The type of mosaic pattern may also be related to the colour mechanism involved in retinal excitation. The structural contact between double and long single cones of the guppy, apart from providing mechanical linkage, may also be a reflexion of functional coupling. Physiological evidence of such coupling has been provided for the turtle and the goldfish[22,23,25]. The cone fins in the guppy may function as synapses, as suggested for the interlocking rod fins of the toad and axolotl[25-27]. Microspectrophotometry of the guppy visual cells has shown that the long single cones absorb maximally at 545 nm and the short single cones at 410 nm. The double cones are either equal, both partners peaking at 545 nm, or unequal with one member having its absorption maximum at 545 nm and the other at 470 nm. The latter member contains, in its ellipsoid, the ellipsosome, which probably acts as a colour filter enhancing contrast detection[14]. However, the composition of the mosaic described above (figure 3) is not uniform over the whole retina: Microscopical analysis shows that short single cones are absent over most of the ventral retina and that the ventral double cones are structurally different from those of the remainder of the retina[13]. Due to the smallness of the guppy eye, microspectrophotometrical analysis does not admit of subdividing the guppy retina in order to determine the origin of the 2 colour types double cones. Therefore, an analysis of the chromatic distribution of the guppy cones over the entire retina, as employed for the goldfish[16], would bring the interpretation of its square mosaic a great step forward.



B

Fig. 2. Electron micrograph of cross-sectioned visual cells in the central retina of the guppy. *A* Square mosaic during light period. The ellipsoids of the zig-zagging double cones and the long single cones are closely appositioned and subsurface cisterns occur along their contact zones. *B* Row mosaic during dark period. Rods have moved vitreally and are sectioned at outer segment level. The contact between the ellipsoids of double cones and long single cones is severed and the subsurface cisterns (c') have disappeared. Short single cones are out of focus because of their vitreal position. Abbreviations: a, accessory outer segment; c, subsurface cistern between partners of double cones; c', subsurface cisterns along contact zone of double cones and long single cones; d, double cone; ls, long single cone; r, rod; s, short single cone.

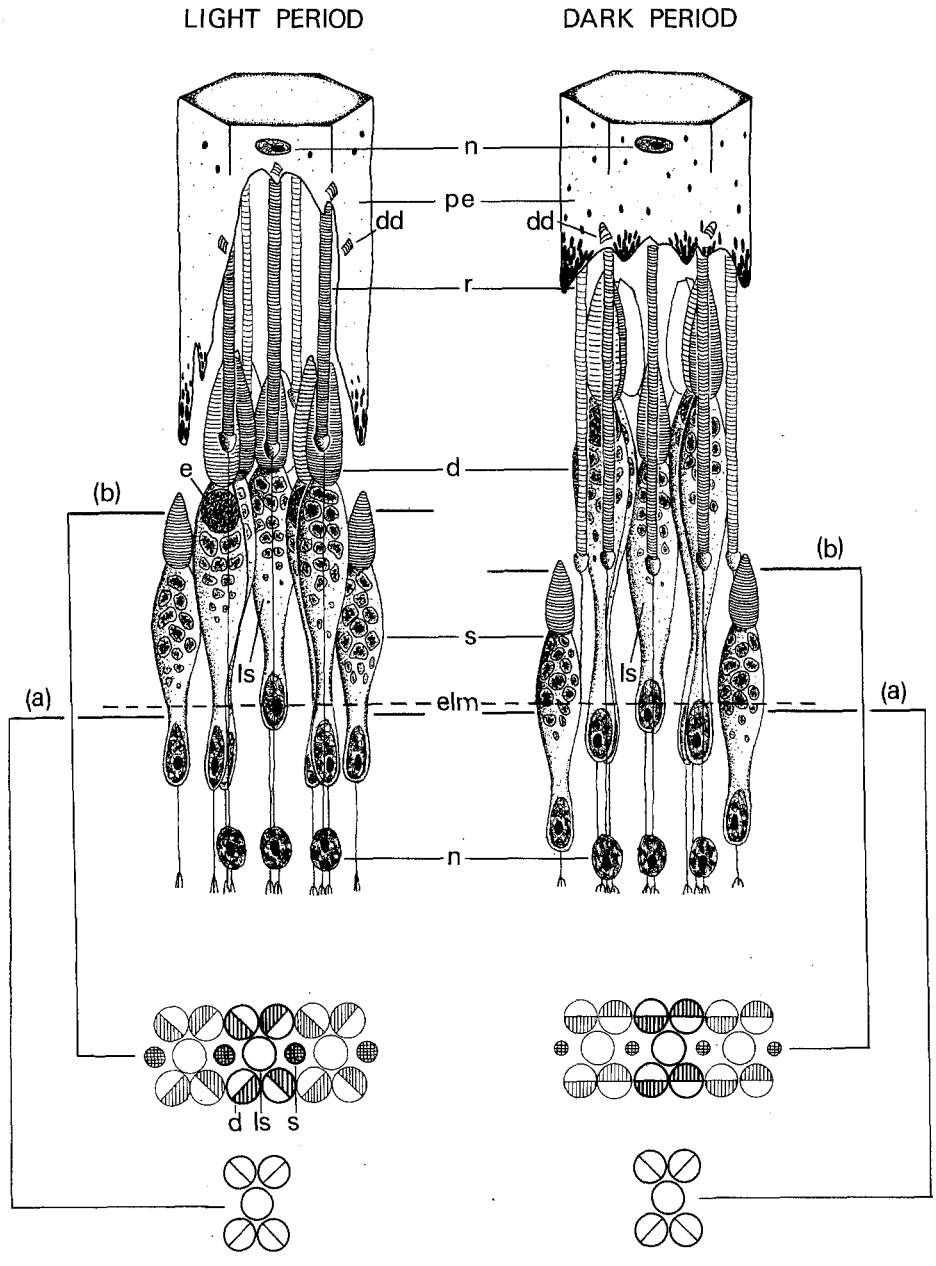


Fig. 3. Semidiagram of retinomotor changes in the guppy. *A* In the light, the extended rods shed their discs into the pigment epithelium and the cones display a square mosaic (a, b). *B* In the dark, cones shed their discs. Scleral to the external limiting membrane double cones twist and assume a row pattern (b) while their nuclei retain the zigzag arrangement (a). Abbreviations: as for figure 2, and dd, discarded discs to be phagocytised by the pigment epithelium – e, ellipsosome; elm, external limiting membrane; n, nucleus; pe, pigment epithelium.

1 Thanks are due to Dr A. Yacob, Biology Department, Mosul University, for supplying the photomicrograph for figure 2A, and to Dr C. Wise, Department of Zoology, University College, Dublin, and B.A. Collins, The Marine Biological Laboratory, Woods Hole, for technical assistance.

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