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REGIONAL SPECIALIZATION OF THE QUAIL RETINA: GANGLION CELL DENSITY AND OIL DROPLET DISTRIBUTION

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The ganglion cell density of the quail's retina was studied in sections and whole mounts. Two regions of high ganglion cell density were found, corresponding to an afoveate area centralis and an area dorsalis. Oil droplets were found to be isotropically distributed throughout the retina. It is proposed that the significance of such retinal regional specialization, in comparison to similar studies in the pigeon and the chick, is that regional specialization in the avian retina is more closely related to feeding habits than to phylogenetic descendence.

Birds are notorious among vertebrates for having two retinal regions of high ganglion cell density: an area centralis and an area dorsalis, located in a more dorso-posterior position but anatomically comparable to the area centralis. Most diurnal predatory birds have a marked fovea in both these regions.

Only recently further details about these specialized regions have been sought, most particularly in terms of quantitative studies of the receptor and ganglion cell density. In the chick retina, a purely terrestrial animal, there is only an afoveate (i.e. an area of high ganglion cell density, but lacking a foveal pit) area centralis [5]. In contrast, the pigeon, an aerial animal, has a distinct afoveate area dorsalis as well. These are the only published quantitative descriptions of ganglion cell density of birds. There is only sketchy evidence for a variety of other species [6, 10].

Yet, data concerning regional specialization in the avian retina are important for an understanding of avian visual abilities since the regions project in a differential manner into the tecto-fugal and thalamo-fugal pathways [4]. Furthermore, in the pigeon, the visual area subtended by the area centralis and dorsalis have two different focal planes and thus seem to play different roles in the animal's behavior [2].

These observations suggest that regional specialization in the avian retina does not correlate well with zoological grouping but rather with habitat and style of food

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gathering. On one extreme one finds the diurnal predatory birds, such as hawks, with their two prominent bifoveate regions, and on the other end of the spectrum, the terrestrial chicken, with its single afoveate area centralis. In between all possible combinations exist: nocturnal predators (owl) with an extensive binocular field and a foveate area dorsalis [4]; the inhabitants of open horizons (albatross) with two areas with shallow foveas and with a stripe of high ganglion cell density running between them [6].

The purpose of this paper is to contribute to this line of study by reporting on the ganglion cell density of the quail retina, an aerial bird in its wild state, but one which has acquired terrestrial habits, although recently, through domestication [9] (see below). This makes it an interesting case to compare with the pigeon and chick.

Quails, as do most birds, have a well-developed color vision [8, 12]. However, there are no quantitative reports describing the possible regional distribution of oil droplets. They participate in color vision by making possible combinations of cone spectral sensitivity beyond those given by the absorption spectra of photopigments [3]. This paper also reports on the oil droplet distribution in the quail's retina as it relates to regional specialization.

Quails (*Couturnix couturnix japonica*) of both sexes were used, purchased from a local dealer and kept in a laboratory animal facility. Only adults were used of an average weight of 120 g. Two different methods were used: (a) on retinal whole mounts and (b) on serial histological sections, and two complete retinas were examined with each method. For whole mounts, the procedure described in ref. 5 was followed, staining with 1% cresyl violet. For counting, the retina was divided in regions of 1 mm^2 through a calibrated grid. For each region, four sub-regions of 5.6×10^{-3} mm^2 were counted directly at $\times 400$. Low-power photomicrographs were used for reconstructions. Animals sedated by ether vapours were perfused through the heart with 10% formalin. The vitreous and bone ring were removed, and gelatin was injected to prevent shrinking of the optic cup. After standard dehydration and inclusion procedures, 10 μm paraffin sections were cut. Orientation was provided by the pecten. Sections were stained with 1% cresyl violet and counting was done every 300 μm, selecting 200 μm regions separated every 400 μm. As sections thicken when cut further away from the geometrical center of the retinal cup, the counting error was estimated following the method used in ref. 7. Because oil droplets are extracted in dehydration procedures, fresh retinas were used. Immediately after excision the retinas were mounted with the receptors pointing upwards. Dark-field microscopy was normally used for counting since this allows direct visualization of all oil droplets. Color photomicrographs were taken every 1 mm^2, and droplets were counted in areas of 0.53 mm^2.

Ganglion cell density. The ganglion cell morphology changed across the retina. In zones of higher density they are smaller in size and are more uniform. In more peripheral regions, they are larger and less uniform in overall shape. There was no apparent foveal pit, neither in fresh retinas nor in sections.

Fig. 1 shows one set of isodensity contours. Both maps are comparable with respect to the geometrical pattern of the ganglion cell gradient and the absolute cellular numbers. We did not pursue in this paper statistics of ganglion cell count along various retinal radii, nor did we attempt to distinguish ganglion cells from glial and amacrine cells (see below). According to the calibrations obtained with small perforations retroprojected with narrow laser beams [8], the maximum cellular counts of the two regions of high density, the area centralis and dorsalis, fell on the horizontal axis of the visual field, at 35° and 90° posterior, respectively. These locations correspond well to similar areas in the pigeon and chick [2, 5]. The maximum cellular density reaches 35 to 40×10^3 cells/mm^2, and these values stand in a 4:1 ratio to the less dense areas in the periphery. Accordingly, the total number of ganglion cells in the quail retina stands at about 1.3×10^6, with an estimated error of 10%.

Oil droplet distribution. The quail retina has four kinds of oil droplets according to size and color: green, orange, red and translucent. They can also be seen in their characteristic locations as inclusions of the retinal cones (Fig. 2).

The oil droplets had an isotropic distribution in these retinas. In all regions we found a density of 25×10^3 droplets/mm^2, with an estimated error of 8%. This uniform distribution is composed of a ratio of 1:5 green, 1:5 orange, 1:5 red, and 2:5 colorless. The total number of oil droplets was 2.5×10^6, a ratio of 2:1 to the total number of ganglion cells. Although the density of oil droplets was constant in this retina, their size clearly co-varied with retinal eccentricity, as shown in Fig. 2. The largest diameters occurred near the periphery, and they were 30% larger than those in the central areas.

Direct inspection of a fresh quail retina revealed a dorsal greenish quadrant. Interestingly, this regional difference was co-related with a high concentration of teardrop-shaped inclusions present in this retina, and which have a yellow-green coloration to transillumination (Fig. 2). As we know of no other reports on these inclusions, their chemical compositions, visual function or if they contribute to the greenish coloration of this area seem to be open questions at the present time.

The quail's retina is comparable to that of the pigeon with regard to the regional pattern and absolute numbers of ganglion cell density. This regional specialization is more marked than in the chick (where only a slightly more dense streak marks the presumptive area dorsalis) but is less marked than in animals such as hawks and kestrels with a bifoveate retina.

In the present study we did not consider the error introduced by the presence of glial cells and displaced amacrines. This error is not negligible. Glial cells amount to about 5–10% in the chick and pigeon; displaced amacrines amount to about 30–35% in the chick and up to 43% in the pigeon [1, 5]. However, although the absolute number of ganglion cells was thereby increased, their relative numbers probably do not change, since at least in the chick the distribution of displaced amacrines is uniform throughout the retina.

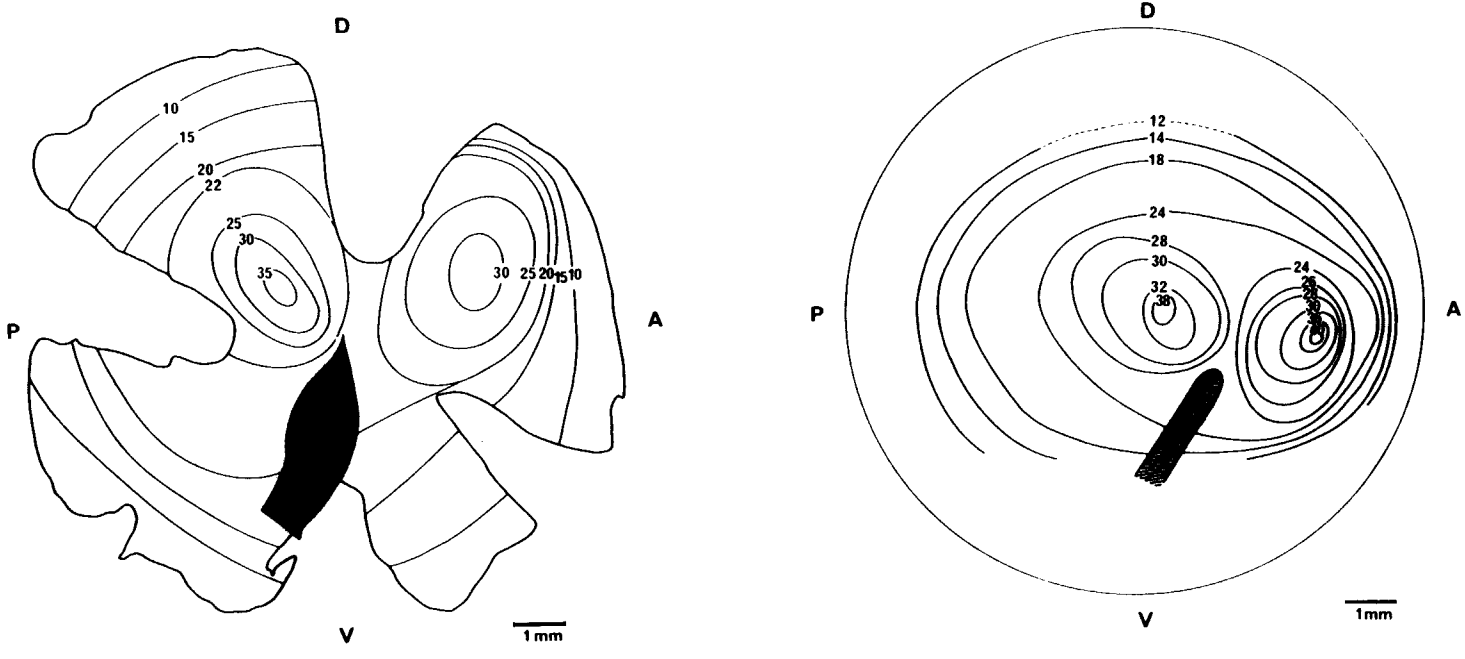

Fig. 1. Iso-density maps of the ganglion cell density of two quail retinae, obtained through whole mounts (left) and sections (right). The numbers should be multiplied by 10^3 to yield cells/mm^2. The hatched area indicates the pecten. A, anterior; P, posterior; D, dorsal; V, ventral.

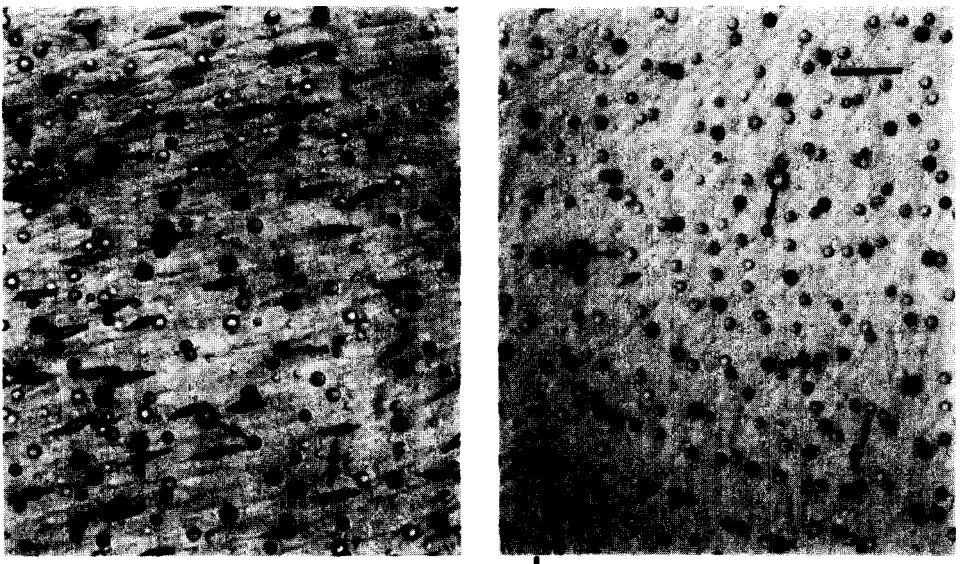

Fig. 2. Light-field image of retinal oil droplets in (a) the periphery or (b) near the central region. Note differences in size and the presence of teardrop inclusions (arrow) found in abundance in the dorsal quadrant of the quail retina. g, green; r, red; o, orange; t, colorless. Bar = 20 μm.

These comparisons suggest that the terrestrial habitat correlates with the loss of the temporal fovea or area dorsalis. Although quails are mostly terrestrial, they have only recently been derived from stocks of predominantly aerial ancestors. In fact, the genus *Couturnix* belongs to a wild type originally from Asia. They have been subject to artificial selection for at least 100 years, but these populations still breed well with the wild type. If left in the wild, these birds have seasonal migrations and terrestrial habits of feeding [9]. Therefore in their ethology quails resemble the pigeon more than the chick, although they are taxonomically more related to the latter (order *Galliformis*, and not *Columbiformes*). Accordingly, their retina has a comparable regional specialization, with two peaks in ganglion cell density.

The uniformity of oil droplet distribution raises several doubts about the role of these structures in color vision. It is surprising that there should not be a higher density of oil droplets in the two areas of high ganglion cell density, precisely the region of highest visual acuity. Also, there is an anisotropic distribution of color responses in the ventral geniculate with blue-sensitive units predominant in number (about 50%) and concentrated in the anterior visual field [8]. The available evidence is insufficient to suggest answers to these questions but shows that the contribution of oil droplets might not be a simple correlate of cone distribution.

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