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COMPARATIVE AND HISTOPHYSIOLOGICAL STUDY OF OIL DROPLETS IN THE AVIAN RETINA

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INDEXING WORDS

colored oil droplets; cone; rod; color vision; wild birds

SYNOPSIS

To investigate the role of the colored oil droplets, we studied the avian retina of 30 species with different ecological habits and different taxonomic positions by microspectrophotometry and histology. Each species had oil droplets of different size and distribution. In diurnal birds, four kinds of colored oil droplets, red, yellow, orange and pale green, were discriminated, whereas in nocturnal birds, only pale-green oil droplets were found.

Cluster analysis of the distribution of droplets demonstrated that the color of the oil droplets in the wild birds correlated with their ecological behavior. The absorption spectra of the red, yellow and orange oil droplets showed only minor interspecific differences. The spectra of the pale green droplets differed significantly between some birds. In the King Penguin, the retina contained pale green droplets only, whose spectrum was completely different from that of other diurnal birds.

These findings suggest that each colored oil droplet has specific advantages for visual perception and each animal has the retina that best matches with its natural environment and feeding habitat. It was also indicated that differences in the characteristics regarding oil droplets reflect the habitat, feeding patterns and breeding patterns of animals.

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INTRODUCTION

Color vision is well developed in many species of fishes, reptiles and birds. However, the color vision is not well developed in mammals except primates. For example, rats, mice, cows and dogs have poor ability of color discrimination, and they have not been used for physiological studies on color vision. The poor color vision of these animals might be attributed in part to their ecological habits. To clarify the possible correlation between animals' capability of color vision and their ecological habits, we studied the oil droplets of retinas of more than 30 species of wild birds having various degrees of color vision.

The presence of oil droplets is a common feature of the cones of many vertebrate retinas, and the oil droplets are sometimes of several colors.[6] Incident light entering the eyeball is received by the cones and the rod cells in the photoreceptor cell layer of the retina. Because the light passes through oil droplets before reaching the outer segments of the cones that mediate color vision,[8,10] the color and size of the oil droplets are thought to have a considerable effect on the function of the cone. Fujimoto,[5] Bowmarker[1] and others have reported that the oil droplets act as a cut-off filter and also as a convex lens.

Ducker,[4] Peiponen,[12] Cullen[3] and Martin[9] have studied the correlation of the color and distribution of oil droplets of birds and suggested that birds with good color vision have oil droplets of several colors. However, previous studies focused primarily on domestic birds and almost no systematic survey has been made of the distribution and absorption spectra of oil droplets in the retinas of wild birds. Present experiment of systematic survey of avian retina of 30 species revealed that the size and distribution in the color of the retinal oil droplets vary according to their ecological habitats.

MATERIALS AND METHODS

Animals

One or three individuals of each of 30 species of birds were used (Table I). These were either captive birds that had been kept in zoos or wild birds that had died in protective custody. Their age and sex were not known.

Tissue preparation and microspectrophotometry

Immediately after death, the eyes were removed and the posterior half of the eye was put in a dish filled with physiological saline supplemented with 1.0 mg/ml of trypsin. Then, the retina was isolated from the pigmented epithelium. The isolated retina was placed on a glass slide with the cones facing upward and observed under a light microscope. The diameter

Table I. List of birds examined in the present study.

Species	No. exsamined
Black-crowned Night Hero *(Nycticorax nycticorax)*	3
Striated Heron *(Ardeola striat)*	2
Little Egret *(Egretta garzetta)*	1
Eastern Turtle Dove *(Streptopelia orientalis)*	1
Japanese Green Pigeon *(Treron sieboldii)*	1
White Eared-pheasant *(Crossoptilon crossptilon)*	1
Golden Pheasant *(Chrysolophus pictus)*	1
Helmet Guineafowl *(Numida meleagris)*	1
Common Turkey *(Meleagris gallopavo domestica)*	1
Pekin Duck *(Anas platyrhyncha domestica)*	3
Black Swan *(Cygnus atratus)*	1
Mandarin Duck *(Aix galericulata)*	2
Black-headed Gull *(Larus ridibundus)*	1
Herring Gull *(Larus argentatus)*	1
Little Cuckoo *(Cuculus poliocephalus)*	1
Magpie *(Pica pica)*	1
Jungle Crow *(Corvus macrorhynchos)*	3
Japanese Lesser Sparrow Hawk *(Accipiter gularis)*	1
Scarlet Ibis *(Eudocimus ruber)*	1
House Swallow *(Hirundo rustica)*	3
Eastern Tree Sparrow *(Passer montanus)*	2
Brown-eared Bulbul *(Hypsipetes amaurotis)*	1
Jungle Nightjar *(Caprimulgus indicus)*	3
Hondo Ural Owl *(Strix uralensis)*	2
Snowy Owl *(Nyctea scandiaca)*	1
King Penguin *(Aptenodytes patagonicus)*	1
Rockhopper Penguin *(Eudyptes chrysocome)*	1
Humboldt Penguin *(Spheniscus humboldti)*	1
Ostrich *(Struthio camelus)*	1
Greater Rhea *(Rhea americana)*	1

of the oil droplets in the central part of the retina was measured and their distribution within one visual field of the microscope was analyzed. In this study we did not analyzed the peripheral retina because the color perception is made mainly at the central retina. As reported by us,[7] density of cone cells is low in the peripheral retina. The morphology of the cone and rod cells was observed with a microscope equipped with differential interference contrast optics (Normarsky optics). The absorption spectrum of each oil droplet was measured with a microspectrophotometer (MPS-50, Shimadzu Corporation). For each oil droplet, absorption at wavelengths from 400 to 800nm was measured five times and the data were averaged. The distribution of colored oil droplets was measured under a light microscope illuminated with either 490nm or 590nm monochromatic light. Under 590nm light, red droplets appeared black, while the others looked transparent. Thus, the number of red droplets was enumerated. When the same field was illuminated with 490nm light, only pale-green droplets looked transparent. By counting the black droplets and transparent ones, we could determine the total number of pale green and/or other (orange and yellow) droplets. Oil droplets in the retinas of the Hondo Ural Owl, Snowy Owl and King Penguin were not measured because they were only of a single color.

Retinal photographs of a live Jungle Nightjar were obtained by funduscopy.

RESULTS

Observation of oil droplets

In the retinas of the diurnal birds, such as Eastern Turtle Dove and the Tree Sparrow (Fig.1A), there were many cones with either red, orange, yellow or pale green oil droplets, and few rod cells. On the other hand, the Black-crowned Night Heron (Fig.1B), which is active in the evening as well as in the day, had more rod cells. Although four colors of oil droplets were present in the Little Cuckoo, most of droplets were orange. Contrasting to them, color of the House Swallow's (Fig.1C) and Jungle Crow's droplets were mostly pale green.

Water birds, such as Night Heron, Pekin Duck and Herring Gull had droplets larger than other birds. The retinas of the Hondo Ural Owl (Fig.1D) and the Snowy Owl have the tapetum lucidum characteristic of nocturnal birds. Their retinas contained many rod cells with long outer segments and cone cells with only pale green oil droplets. The retina of the King Penguin was similar to that of nocturnal birds, but the retinas of the Humboldt Penguin and Rock-hopper Penguin contained four colors of oil droplets similar to those of diurnal birds.

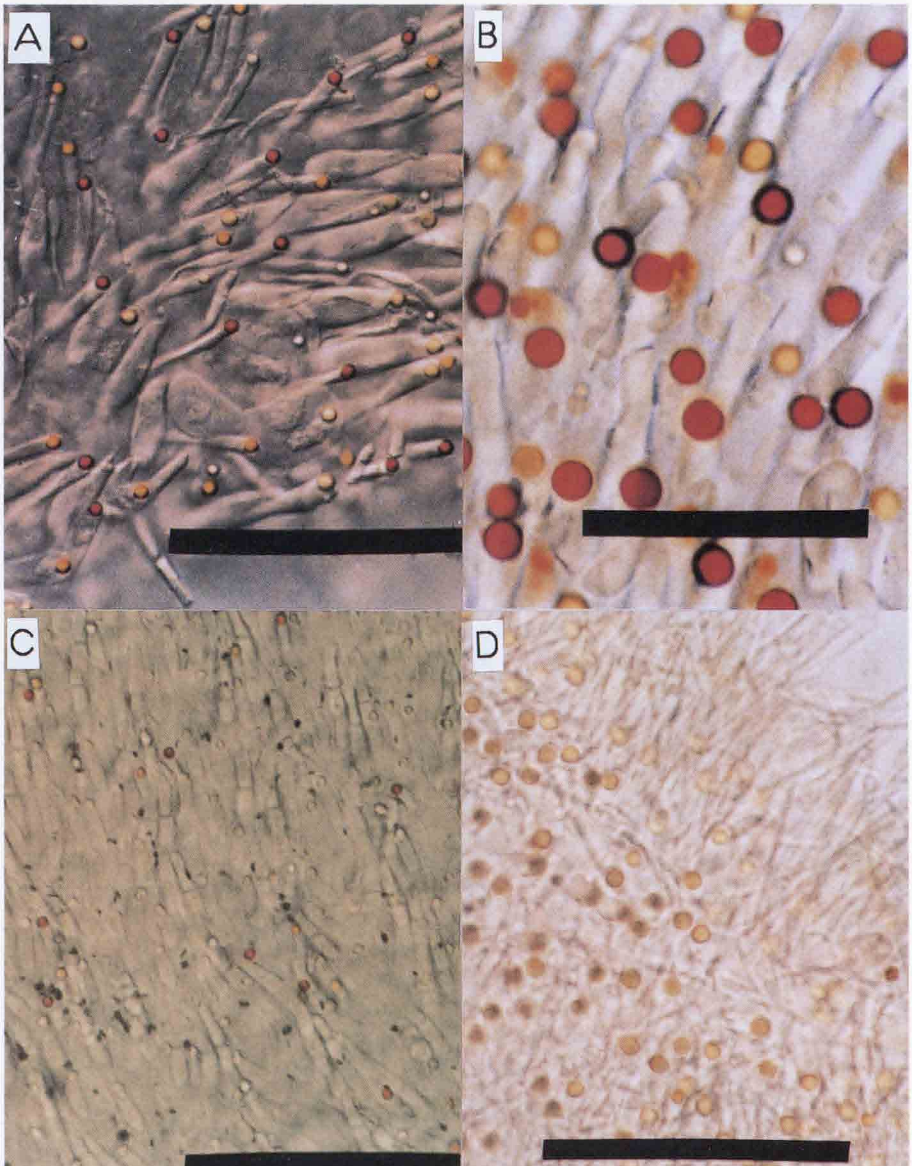

Fig. 1. Retinas of wild birds examined by Normalsky optics. A: Retina of *Passer montanus*. Note the four colors of oil droplets. Bar= 25μm. B: Retina of *Nycticorax nycticorax*. Bar= 50μm. C: Retina of *Hirundo rustica*. Bar= 25μm. D: Retina of *Strix uralensis*. Bar= 50μm.

In the Jungle Nightjar, which is active in the evening, the upper half of the retina looked silver-gray and the lower half appeared black. To confirm that this unusual pigmentation was not an artifact, we examined the retina of a living Jungle Nightjar by a funduscopy. Only the upper half of the retina possessed the tapetum. The cone cells of the Jungle Nightjar contained oil droplets of four colors similar to those of diurnal birds.

Table II summarizes the size and the distributional density of oil droplets found in the retinas of 13 species.

Absorption spectrum of oil droplets

We analyzed the absorption spectra of the oil droplets in the retinas of the Black-crowned Night Heron, Jungle Crow, Eastern House Swallow, Jungle Nightjar and King Penguin by microspectrophotometry (Figs.2~6). The concentration of carotenoids in the deeper-colored droplets was very high, with peak absorption coefficients greater than 10. However, the actual peak absorption coefficients were only about 1.0, indicating a significant influence of stray light on peak absorption. Therefore, we characterized the droplets only by the wavelength at 50% transmission and by the slope of the curve in the interrupted region.

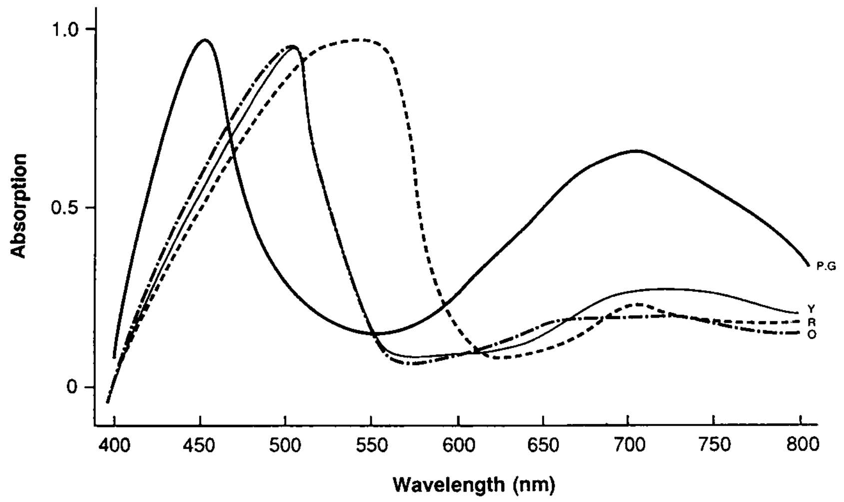

Fig. 2. Absorption Spectra of the oil droplets of *Nycticorax nycticorax*. R: red oil droplet; O: orange oil droplet; Y: yellow oil droplet; PG: pale green oil droplet.

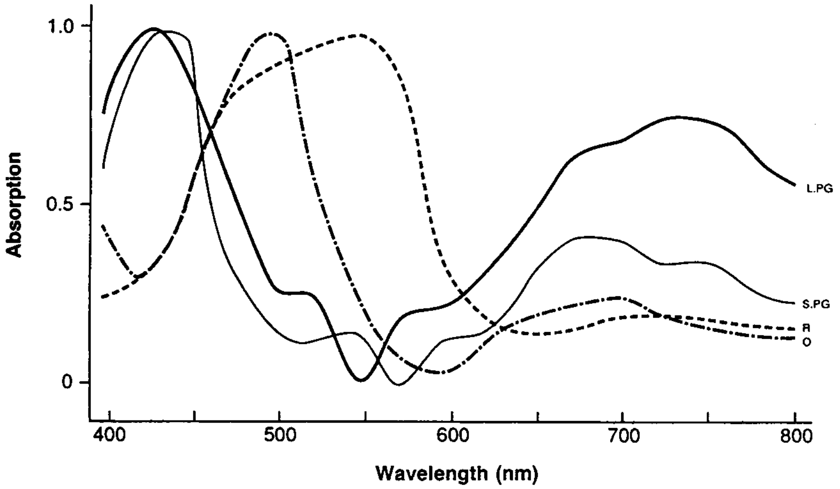

Fig. 3. Absorption Spectra of the oil droplets of *Corvus macrorhynchos*. LPG: large pale green oil droplet; SPG: small pale green oil droplet.

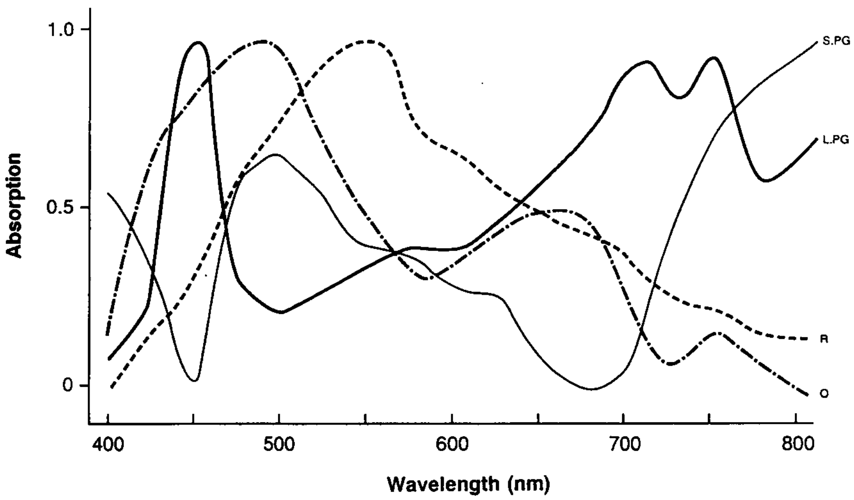

Fig. 4. Absorption Spectra of the oil droplets of *Hilundo rustica*.

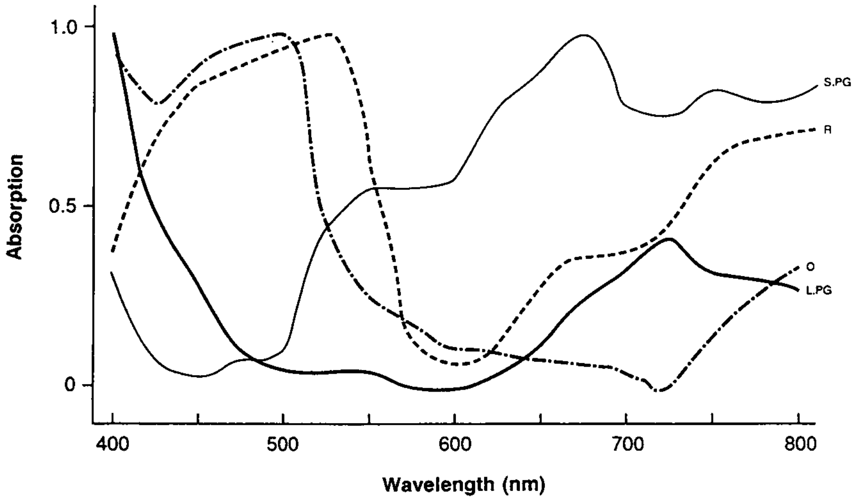

Fig. 5. Absorption Spectra of the oil droplets of *Caprimulgus indicus.*

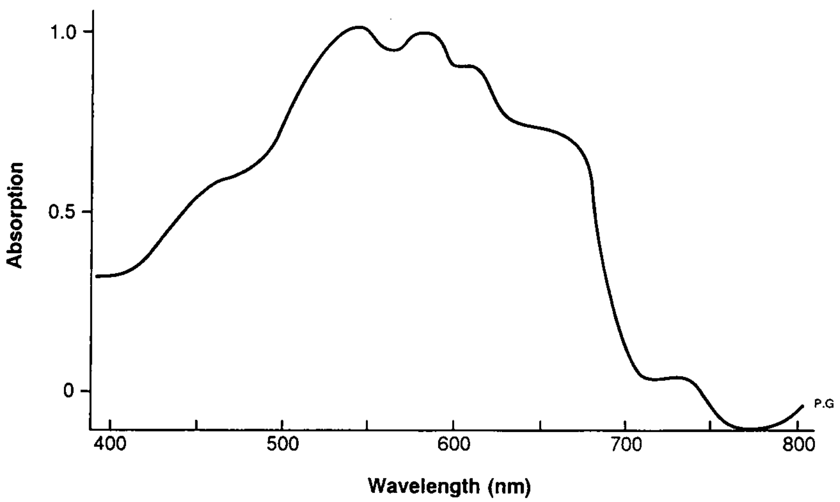

Fig. 6. Absorption Spectra of the oil droplets of *Aptenodytes patagonicas.*

Table II. The size and relative abundance of the oil droplets in the retina of 13 avian species.

Color of oil droplets	Red		Yellow, Orange		Pale green	
Species	D(μm)	%	D(μm)	%	D(μm)	%
Nycticorax nycticorax	5~7	18	5	31	2	51
Passer monotnus	4	31	3.5	36	5~3	33
Streptopelia orientalis	4	26	3.5	38	4	36
Anas platyrhyncos domestica	5	23	5.5	39	4	38
Larus argentatus	4	26	4	38	3	36
Cuculus poliocephalus	3	14	4	41	2	45
Corvus macrorhynchos	4	17	3	23	4~2.5	60
Pica pica	4	20	4	22	5~3	58
Hirundo rustica	2	12	2	10	2	78
Caprimulgus indicus	2	16	2	32	5~ 2	52
Strix uralensis					3~2	100
Nyctea scandiaca					3~2	100
Aptenodytes patagonica					3~2	100

D:diameter.

%:relative abundance.

DISCUSSION

We studied the absorption spectra of oil droplets in the retinas of the Black-crowned Night Heron, Jungle Crow, Eastern House Swallow, Jungle Nightjar and King Penguin by microspectrophotometry. Red oil droplets were characterized as a color filter with cut-off wavelength of 580nm, yellow and orange oil droplets had shorter cut-off wavelength of 530nm. There were no major differences in these values among the species examined. However, the absorption spectra of pale green oil droplets differed greatly from species to species. The absorption spectrum of the King Penguin, which had only pale green oil droplets, was entirely different from that of all the other species examined. It indicates that the oil droplets of this species contain unique carotenoid.

According to the study by Muntz[11] on seabirds, the proportion of red and orange oil droplets is approximately from 50% to 60% in light-feathered birds, whereas that in dark-feathered birds is as low as 20%. In dark-feathered birds, the majority of droplets are pale green. We analyzed the proportion of oil droplets of different colors in 13 species of the birds used in this study (see Table II). In the light-colored Pekin Duck, Black-crowned Night Heron, Tree Sparrow and Herring Gull, the proportion of red oil droplets plus orange and yellow was in the range of 49% to 67%. In the dark-colored Little Cuckoo, Jungle Crow, Magpie, Eastern House Swallow and Jungle Nightjar, the proportion of pale green oil droplets was high (45%~78%), being close to the value reported by Muntz.[11]

As reported by Braekvelt,[2] the Great Horned Owl, *Bubo virginianus*, has only pale green oil droplets. We found that the same is the case with the Hondo Ural Owl, the Snow Owl and the King Penguin.

In order to analyze the similarities of oil droplets among 10 species of wild birds, we drew a dendrogram by applying the farthest neighbor method of cluster analysis to the data of table II, (Fig.7). As shown in the figure, the Eastern House Swallow, *Hirundo rustica* and the Jungle Nightjar, *Caprimulgus indicus*, both are insectivores, stand apart from the other groups. The dendrogram also suggests a close similarity between the Black-crowned Night Heron, *Nicticorax nicticorax* and the Pekin Duck, *Anas platyrhyncas domestica.* The Tree Sparrow, *Passer montanus* and the Eastern Turtle Dove, *Streptoelia orientalis*, also grouped together; they eat seeds and insects on the ground or in bushes. Thus, each group has similar habitat and feeding behavior. These results seem to indicate that the color of the retinal oil droplets in the wild birds correlates with their ecological habits.

The retina of the Jungle Nightjar presented a unique structure. It is well known that this bird flies over places where mosquitoes swarm in columns in early evening. As the sky

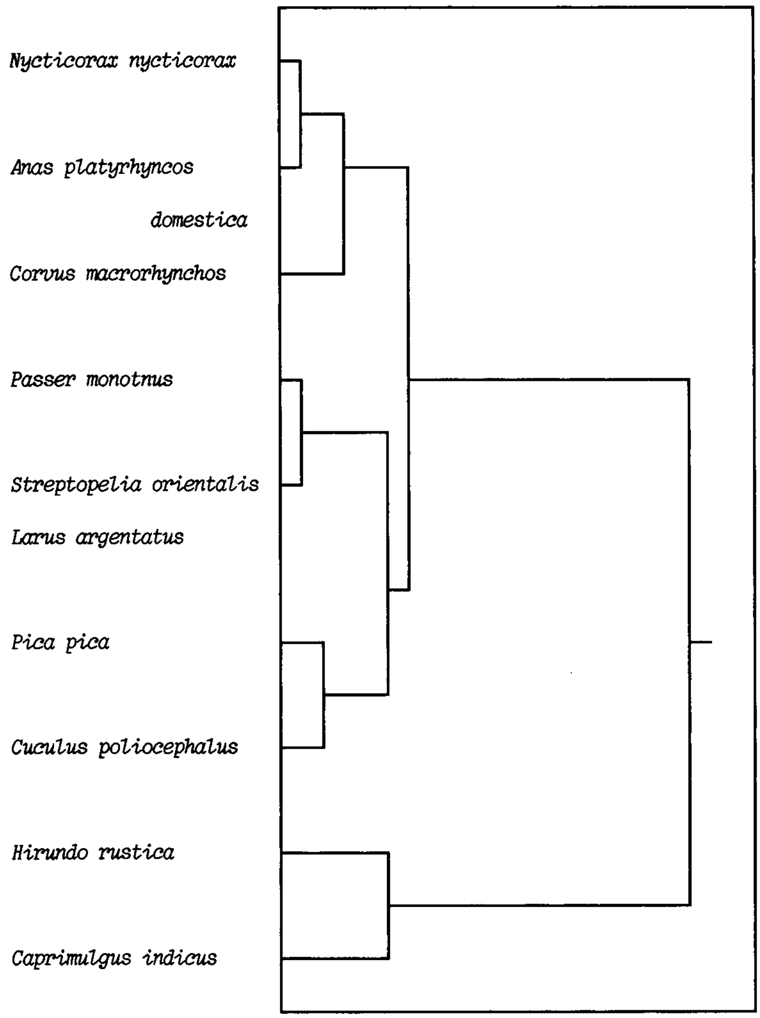

Fig. 7. Dendrogram of the similarities among oil droplets of 10 avian species. The dendrogram was obtained by farthest neighbor method of cluster analysis.

over such places at that time of the day is still bright, the Jungle Nightjar would perceive images from above with the lower half of its retina, whose morphology is similar to that of diurnal birds. It perceives darkened images from below by the upper half of the retina, where it has tapetum as in nocturnal birds.

Based on the absorption spectra, we presume that the red oil droplets in the retina serve to make red fruit conspicuous by absorbing the light from green leaves. In fact, the Brown-eared Bulbul and Eastern Turtle Dove, which habitually eat red fruit, had a large number of red oil droplets. Peiponen[12] reported that the proportion of red oil droplets is large in birds that fly over water or watch the water surface. In gulls, the proportion of red oil droplets was approximately 80% in species that fly over water, while the proportion was approximately 60% in species like Black-headed Gull that spend considerable amount of time inland. This may be in part due to the fact that the red oil droplets function to reduce flashes and glare.[13] The present data support this assumption. The retinas of the Black-crowned Night Heron, Pekin Duck and Herring Gull contained many large red oil droplets. The Black-crowned Night Heron and other herons can pursue animals under the water despite the glare from the surface, probably because the oil droplets serve to prevent the dazzling effect of the glare. It may also be possible that the absorption characteristics of each oil droplets are used as a glare-prevention filter.

Penguin is classified into one family, six genuses and 18 species. The retina of King Penguin contained only pale green oil droplets and many rod cells as in nocturnal bird. This probably reflects the fact that it lives in a world void of color in the Antarctic region. The visual system of King penguin seems to well adopted to get foods in the dark sea of Antarctic region. On the other hand, the retinas of the Humboldt Penguin living in a color filled world near the Equator and the Rock-hopper Penguin living in areas with vegetation, although close to the polar region, contained oil droplets of four colors.

As is shown by the study on the penguins, there can be great differences in the properties of oil droplets even among closely related species. Similar differences were also present in gull and doves.[7] Therefore, we may conclude that phenotype of the retinal oil droplets is highly variable due to their ecological habits.

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