HISTOLOGICAL BASIS OF AGE-RELATED CHANGES IN IRIS COLOR IN THE AFRICAN PIED STARLING (*SPREO BICOLOR*)

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ABSTRACT.—Juvenile African Pied Starlings (*Spreo bicolor*) have a dark brown iris, but after the first year iris color changes progressively until adult birds have a creamy-white iris. Using light and electron microscopy, we found that the pigment epithelium of the iris remains pigmented, while changes occur in the pigmentation of the anterior border layer and the stroma of the subadult and adult iris. In the juvenile iris the anterior border layer is darkly pigmented, but in the subadult iris this pigmentation is lost progressively, while pigment granules are deposited in the stroma. In adults, the stroma is heavily pigmented with a solid refractory pigment, and the anterior border layer is clear. *Received 12 January 1990, accepted 12 July 1990.*

MOST passerine birds have a dark-colored iris, as opposed to a red, yellow, or white iris. Iris color can be an aid to species identification, and banders have used variations in iris color to age individuals (e.g. Selander 1958, Wood and Wood 1972, Brensing 1985). In some species iris color varies geographically (Mayr and Vaurie 1948, Selander 1958, Wilkinson 1984), whereas other species may be polymorphic for different iris colors (Pearson 1966). Changes in iris color with age have been described in detail for a few species (e.g. Selander 1958, Hardy 1973), but the morphological basis of such changes has received little attention.

Balducci (1905) examined the histology of the iris in relation to pigmentation in the Little Owl (*Athene noctua*), and Bond (1919) described the anatomical basis of different iris colors in domestic fowl and pigeons. With light microscopy and chemical tests to identify the pigments in iris tissue, Oehme (1969a) surveyed a wide range of families. Several histological studies of the avian iris have concentrated on the musculature (e.g. Oehme 1969b, Oliphant et al. 1983, Fischer and Dieterich 1985), and others have focused on the pigment cells (e.g. Ferris and Bagnara 1972, Oliphant 1981). Further information on the chemical identification of pigments in the avian iris is provided by Oliphant (1987a, b; 1988).

The African Pied Starling (*Spreo bicolor*) is a common open-country species endemic to South Africa (Craig 1985). The birds are colonial cooperative breeders (Craig 1987) and flock throughout the year. Age classes can be distinguished in the field by iris color and plumage characteristics. We compared the morphology of the iris in birds of different ages.

MATERIALS AND METHODS

Specimens of juvenile, subadult, and adult African Pied Starlings were collected on Table Farm (33°16'S, 26°25'E) near Grahamstown, South Africa. The irises were fixed in situ by immediate injection of Bouin's fixative (for light microscopy) or 2.5% phosphate-buffered glutaraldehyde (for electron microscopy). The irises were then dissected and prepared.

Light microscopy.—Fresh irises were processed routinely (Humason 1967), mounted in paraffin, sectioned and stained with hemotoxylin and eosin. We made transverse sections of the whole iris, but only those that passed through the center of the pupil were used for comparisons.

Electron microscopy.—Freshly fixed irises were dissected into thin radial strips. These were fixed in 2.5% phosphate-buffered glutaraldehyde at 4°C for 7 hours. Post-fixation was in 2% osmium textroxide in phosphate buffer. Finally the iris tissue was dehydrated in an ethanol series and infiltrated in an araldite/taab resin mixture for approximately 8 h at each dilution (Cross 1985). The tissue was polymerized in resin molds at 60°C, and sectioned on an LKB UM3 ultramicrotome with glass knives. The sections were mounted on uncoated copper grids, stained with uranyl acetate and lead citrate, and viewed under a JEOL 100 CX transmission electron microscope.

RESULTS

Juvenile birds have a dark brown iris in the first year (Fig. 1a). Subadult birds are distinguished by a progressive increase in the creamy-

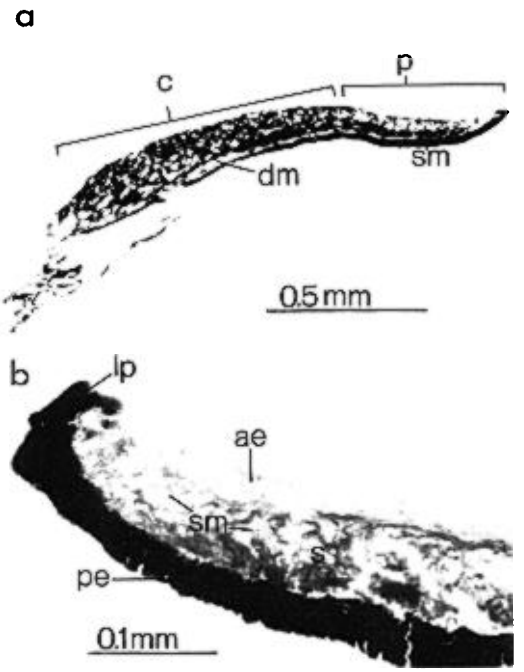

Fig. 2. Structure of the iris: (a) whole adult iris and (b) pupillary region of an adult iris. Abbreviations: ae = anterior border layer; c = ciliary region; dm = dilator muscle; lp = pupillary lip; p = pupillary region; pe = pigment epithelium; s = stroma; and sm = sphincter muscle.

white area, which first appears peripherally on the iris (Fig. 1b). The adult has a completely white iris (Fig. 1c). There is some individual variation, but generally 2-year-old birds have the adult condition.

Light microscopy.—The iris is divisible into the pupillary region, defined by the presence of the sphincter muscle, and the ciliary region, which extends from the proximal side of the sphincter muscle to the junction with the ciliary muscle (Fig. 2a).

In transverse section, there are three distinct layers (Fig. 2b). Posteriorly there is a double layer of pigmented epithelium. The dilator muscle is associated with the anterior part of this epithelium. The major component of the iris is the stroma, a loose arrangement of cells and ground substance interspersed with colla-

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Fig. 1. African Pied Starling (*Spreo bicolor*) (a) juvenile with dark eye, (b) subadult with peripheral creamy-white ring in the iris, and (c) adult with completely white-colored iris.

gen fibers and striated muscle. At the anterior margin is the anterior border layer. At the pupillary margin of the iris, the pigment epithelium forms a lip and extends a short distance on the anterior surface.

In the juvenile iris the anterior border layer is darkly pigmented (Fig. 3a), but the extent of this dark pigmentation is reduced in the subadult (Fig. 3b). The pigment is progressively lost from the ciliary margin inwards towards the pupil. The anterior border layer of the adult iris lacks dark pigment, but contains a small amount of the same pigment that develops in the stroma (Fig. 3c).

We distinguished two cell types in the juvenile stroma. Fibrocytes, which have smaller, round, red-staining nuclei, are less numerous than the larger, oval pigment cells with dark nuclei (Fig. 4a). The cells in the juvenile stroma have a relatively clear cytoplasm, and they transmit light readily. However, in the adult and subadult the stroma is dominated by pigment cells whose cytoplasm appears yellow-brown in transmitted light (Fig. 4b). In reflected light, the sectioned stroma appears creamy-white. The change again proceeds from the ciliary margin inwards. At all ages, the pigment epithelium remains pigmented darkly (Fig. 5).

Electron microscopy.—The pigment epithelium has a similar structure in all three age classes (Fig. 6). It consists of a double layer of cells: the outer layer is columnar, and the inner layer cuboidal. The cells are all heavily pigmented with inclusions that resemble melanin granules. The granules are distributed evenly throughout the cytoplasm. The pigment granules are oval, with their axes arranged randomly.

The stroma consists of ground substance, interspersed with irregularly arranged bundles of collagen fibers (which appear to be orientated randomly), striated muscle, and other cells (Fig. 7). In juveniles, the stroma has relatively large intercellular spaces, filled mostly by pigment cells in adults. These stromal pigment cells contain numerous recognizable artifacts of hard, crystalline reflecting platelets (cf. Ferris and Bagnara 1972, Oliphant 1981). These reflecting platelets are solid and either fall out or are dissolved during processing (cf. Oliphant 1987a). Characteristic holes remain in the tissue (Fig. 8a). The stromal pigment cells of juvenile birds lack this feature (Fig. 8b). In adult African Pied Starlings the stromal pigment cells contain only

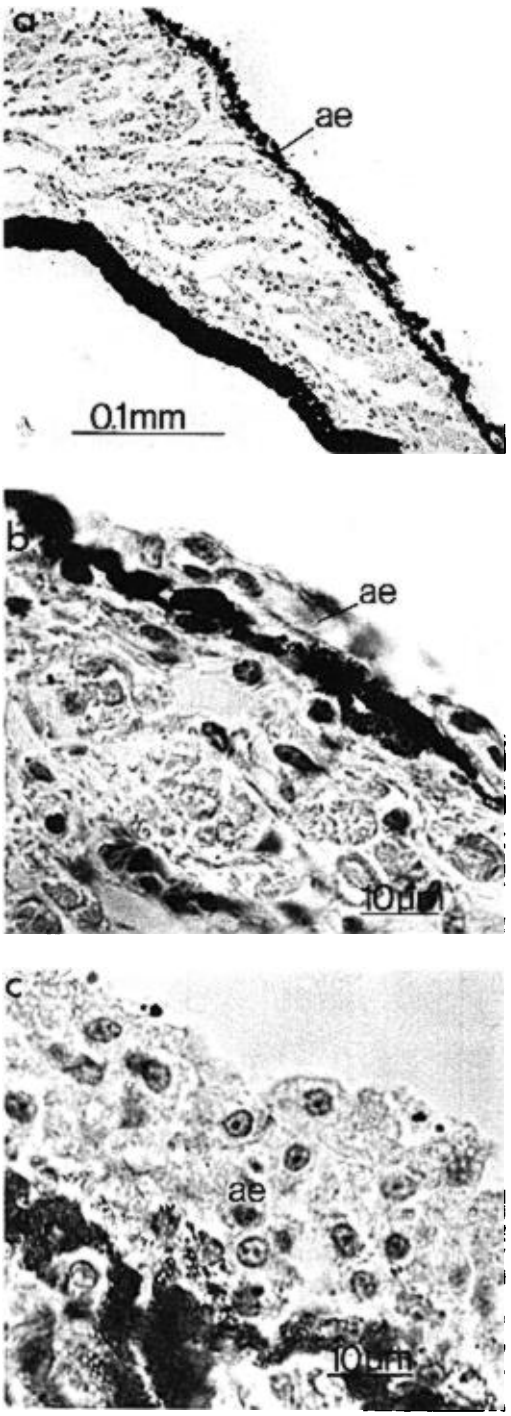

Fig. 3. Sections through anterior border layer: (a) juvenile, (b) subadult, and (c) adult (ae = anterior border layer).

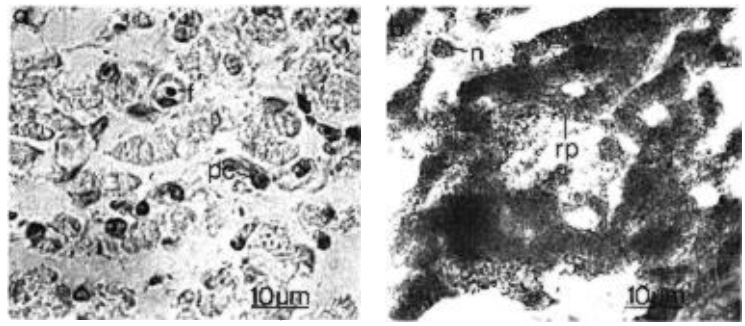

Fig. 4. Light micrographs of the iris stroma: (a) juvenile condition and (b) adult condition. Abbreviations: f = fibrocyte; n = nucleus; pc = pigment cell; and rp = reflecting platelets.

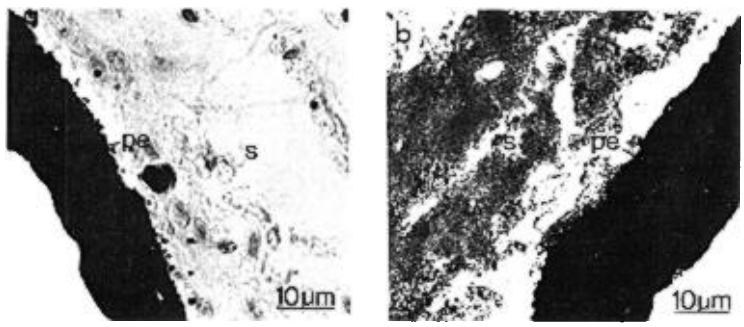

Fig. 5. Sections through the pigment epithelium: (a) juvenile and (b) adult. Abbreviations: pe = pigment epithelium; s = stroma.

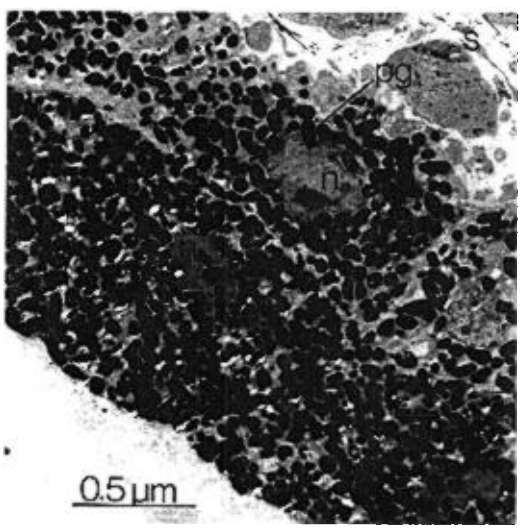

Fig. 6. Electron micrograph of pigment epithelium. Abbreviations: n = nucleus; pg = pigment granule; s = stroma.

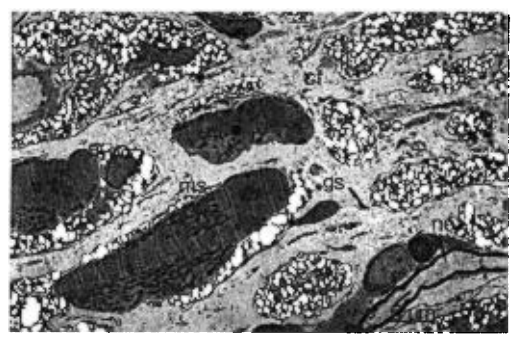

Fig. 7. Electron micrograph of adult stroma. Abbrevations: cf = collagen fibers; gs = ground substance; ms = striated muscle; ne = possible nerve fiber; pc = pigment cell.

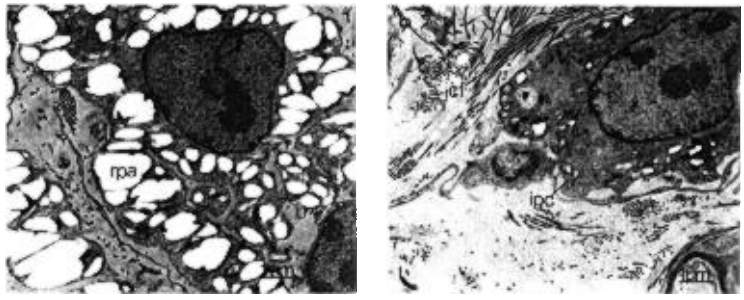

Fig. 8. Electron micrographs of pigment cells: (a) pigment cell from adult stroma and (b) pigment cell from juvenile stroma. Abbreviations: cf = collagen fibers; ipc = pigment cell of immature bird; n = nucleus; rpa = reflecting platelet artifact.

reflecting platelets, although in the subadult, melanin granules and reflecting platelets occur together in the some cells. No pigmented granules were present in the pigment cells of juvenile birds. Two other cell types recognizable in the stroma are elongate fibrocytes, with variably shaped nuclei, and mast cells. The mast cells are rarer, and characterized by cytoplasmic extensions and relatively large, round nuclei.

The anterior border layer consists of closely packed stromal cells. In the juvenile and to a lesser extent in the subadult, the anterior border layer contains large numbers of melanin granules (Fig. 9a). However, in the adult there are no such granules, but there are some reflecting platelets as described in the stroma (Fig. 9b).

Discussion

Balducci (1905) examined sections of the iris from the yellow-eyed Little Owl (*Athene noctua*) and a brown-eyed juvenile Short-eared Owl (*Asio flammeus*). He noted that in both species there was a posterior pigmented region that contained melanin granules, but the Little Owl also had granular pigment in the anterior section of the iris, which he termed a lipochrome layer. The Short-eared Owl lacked this layer. Bond (1919) proposed that dark irises in birds could have two different structures: (1) a lack of pigment in the anterior border layer and stroma, so that the dark pigment epithelium determined the color; and (2) dark pigment in the anterior border layer as well as in the pigment epithelium. The juvenile condition in the Pied Starling is clearly an example of the second structure. Fischer and Dieterich (1985) interpreted the iridal pigment cells of the Black-billed Magpie (*Pica pica*) as myoepithelial cells, and Oliphant et al. (1983) came to the same conclusion for the Great Horned Owl (*Bubo virginianus*).

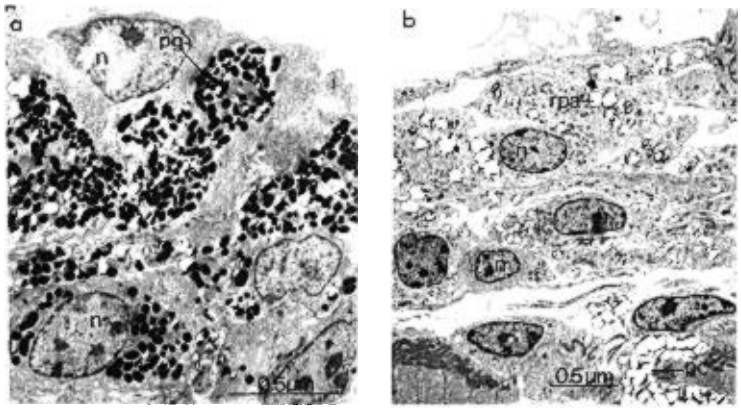

Fig. 9. Micrographs of the anterior border layer: (a) juvenile and (b) adult. Abbreviations: n = nucleus; pc = pigment cell; pg = pigment granule; rpa = reflecting platelet artifact.

Oehme (1969a) found a posterior pigment epithelium containing melanin in all 140 species he examined, and he distinguished 32 iris types on the basis of the pigments present and their distribution. Oehme (1969a) noted that there was little indication that particular pigments or structural arrangements were restricted to certain taxa. However, in all three Sturnidae examined (the European Starling, *Sturnus vulgaris*; the Crested Myna, *Acridotheres cristatellus*; and the Common Myna, *A. tristis*) pteridines were present in both the outer layer of the pigment epithelium and in the stroma. This was not the case in the Pied Starling, which is not closely related to the Asian genera *Sturnus* and *Acridotheres* (Sibley and Ahlquist 1984).

The stromal pigment cells of the Pied Starling closely resemble those figured by Ferris and Bagnara (1972) and Oliphant (1981, 1987a). We have not tested the composition of the pigment granules, but it is likely that pteridines are present (cf. Oliphant 1987b, 1988). Oehme (1969a) found pteridines in both *Sturnus* and *Acridotheres*, and in the European Starling and the Common Myna some cells contained both melanin and pteridines. We found stromal pigment cells that contained two pigment types in subadult Pied Starlings.

Age-related changes in the iris tissue of the Herring Gull (*Larus argentatus*) were described by Bond (1919). In the nestling there was a plexus of dark-pigmented cells on the anterior surface of the iris. This was retained in the female, but in the adult male these dark cells appeared to lie just above the pigmented epithelium, while a layer of cells with yellow pigment was found in the anterior border layer. Bond (1919) proposed that the color change was the result of cell migration. Andrew and Naik (1965) found that in the nestling Jungle Babbler (*Turdoides striatus*) the dark color was caused by the dark pigment epithelium, which was visible through the thin transparent muscle layer and stroma. In older birds more numerous muscle fibers made the iris opaque and cream-colored. Finally in the adult bird yellow pigment was deposited in the stroma.

In the African Pied Starling, as in the Herring Gull, dark pigment in the anterior border layer is replaced by reflecting pigment granules, which also appear in the stromal cells. The muscle layer does not play a significant role in this change. Presumably the pigment content of individual cells changes, instead of cells migrating. This is supported by the presence of fluorescent materials in the iris cells of immature pigeons before the development of the yellow stromal cells typical of the adult (Oliphant 1987a). Further studies of the development of iris pigmentation, and its relationship to other physiological processes such as sexual maturation, are needed.

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LITERATURE CITED

ANDREW, M. I., & R. M. NAIK 1965. Structural basis of the change of eye colour of the Jungle Babbler, *Turdoides striatus* (Dumont), during post-embryonic development. Pavo 3: 72–74.

BALDUCCI, E. 1905. Osservazioni e considerazioni sulla pigmentazione dell'iride dell' *Athene chiaradiae* Gigl. Monit. Zool. Ital. 16: 258–272.

BOND, C. J. 1919. On certain factors concerned in the production of eye colour in birds. J. Genetics 9: 69–81.

BRENSING, D. 1985. Alterskennzeichen bei Sumpf- und Teichrohrsanger (*Acrocephalus palustris, A. scirpaceus*): quantitative Untersuchung. J. Ornithol. 126: 125–153.

CRAIG, A. J. F. K. 1985. The distribution of the Pied Starling, and southern African biogeography. Ostrich 56: 123–131.

———. 1987. Cooperative breeding in the Pied Starling. Ostrich 58: 170–180.

CROSS, R. H. M. 1985. The preparation of biological material for electron microscopy. Grahamstown, South Africa, Rhodes Univ.

FERRIS, W., & J. T. BAGNARA. 1972. Reflecting pigment cells in the dove iris. Pp. 181–192 *in* Pigmentation: its genesis and biological control (V. Riley, Ed.). New York, Meredith.

FISCHER, G., & H. J. DIETERICH. 1985. Feinstrukturelle Befunde an der glatten Muskulatur in der Iris der Elster (*Pica pica*). Z. mikrosk.-anat. Forsch., Leipzig 99: 415–424.

HARDY, J. W. 1973. Age and sex differences in the black-and-blue jays of middle America. Bird-Banding 44: 81–90.

HUMASON, G. L. 1967. Animal tissue techniques. San Francisco, W. H. Freeman.

MAYR, E., & C. VAURIE. 1948. Evolution in the family Dicruridae (birds). Evolution 2: 238–265.

OEHME, H. 1969a. Vergleichende Untersuchungen

uber die Farbung der Volgeliris. Biol. Zentralbl. 88: 3-35.

———. 1969b. Der Bewegungsapparat der Volgeliris (Eine vergleichende morphologisch-funktionelle Untersuchung). Zool. Jahrb. Anat. 86: 96-128.

OLIPHANT, L. W. 1981. Crystalline pteridines in the stromal pigment cells of the iris of the Great Horned Owl. Cell Tissue Res. 217: 387-395.

———. 1987a. Observations on the pigmentation of the pigeon iris. Pigment Cell Res. 1: 202-208.

———. 1987b. Pteridines and purines as major pigments of the avian iris. Pigment Cell Res. 1: 129-131.

———. 1988. Cytology and pigments of non-melanophore chromatophores in the avian iris. Adv. Pigment Cell Res. 1: 65--82.

———, M. R. JOHNSON, C. MURPHY, & H. HOWLAND. 1983. The musculature and pupillary response of the Great Horned Owl iris. Exp. Eye Res. 37: 583-595.

PEARSON, D. J. 1966. Observations on the iris colour of Bearded Tits. Bird Study 13: 328-330.

SELANDER, R. K. 1958. Age determination and molt in the Boat-tailed Grackle. Condor 60: 355-376.

SIBLEY, C. G. & J. E. AHLQUIST. 1984. The relationships of the starlings (Sturnidae: Sturnini) and the mockingbirds (Sturnidae: Mimini). Auk 101: 230-243.

WILKINSON, R. 1984. Variation in the eye colour of Blue-eared Glossy Starlings. Malimbus 6: 2-4.

WOOD, D. L., & D. S. WOOD. 1972. Numerical color specification for bird identification: iris color and age in fall migrants. Bird-Banding 43: 182-190.