PIGMENTATION AND FEATHER STRUCTURE IN GENETIC VARIANTS OF THE GOULDIAN FINCH, *POEPHILA GOULDIAE*

ALAN H. BRUSH

AND

HAROLD SEIFRIED

THE Gouldian Finch, *Poephila gouldiae*, is among the most colorful of estrildine finches. The species is distributed through tropical northern Australia, where it inhabits grassy plains. It is nomadic over at least part of its range, is an extremely sociable species, and occurs in large flocks even during the breeding season. Because of its colorful plumage and simple diet of seeds, it enjoys considerable favor as a cage bird.

One of the most interesting aspects of the plumage in this species is the occurrence in nature of a polymorphism in facial color. Three facial or head colors are known in wild birds: black, which is the most common; red, which occurs in approximately one out of four birds; and yellow or orange, which occurs in only one in every three to five thousand birds. As the color phases are known from all areas of the species' range and occur in all flocks, they are not considered separate geographic races. In addition to the three face colors, there are several mutants that involve both melanistic pigmentation (Butler, 1902) and feather structure (Immelmann, 1965, pers. comm.) but these are only poorly known.

Because the Gouldian Finch is popular as a cage bird, a considerable amount of information has been obtained on the genetics of the facial colors. Southern (1946) has shown that although the black type is more common than the red, the red is dominant over black. Furthermore, the red and black alleles are sex linked. Murray (1963) showed that the gene for the rarer orange face is autosomal and recessive to both red and black. Birds that are homozygous for yellow and also for the recessive black head are black faced but have a yellow tipped beak rather than the usual red tip (Southern's "type C" black genotype).

We undertook the investigations on the pigmentation and feather structure reported here for several reasons. The Gouldian Finch represents an opportunity to study carotenoid metabolism in a nondomestic species about whose genetics some information is available. Relatively little is known about the metabolism of these pigments in vertebrates generally and, in spite of the wide use of plumage coloration in avian systematics, practically no information is available on the pathways of carotenoid metabolism in birds. *P. gouldiae* is of potential general interest in an attempt to understand the control and evolution of these metabolic pathways. This finch also provides ideal material for studies on the metabolism of pigments in

birds with complex plumage patterns. Much of the previous work on the biochemistry of pigments has been with species that are essentially monochromatic in their carotenoid patterns (Fox and Hopkins, 1966*a*; Völker, 1962). Finally, this material allowed us to extend considerably some earlier work on the relationship between pigment metabolism, deposition, and feather structure.

Materials and Methods

Skins of the three color phases of the brightly colored, highly patterned Gouldian Finch were generously provided by H. B. Tordoff of the University of Michigan Museum of Zoology. Adults of both sexes are similarly colored, with the females somewhat duller. The back and upper surfaces of the wings are green, the rump and upper tail coverts cobalt-blue, the rectrices black, the foreneck and breast lilac with a caudal margin of yellow-orange. The abdomen and sides are yellow, the lower central abdominal area and under tail coverts white, the bill is greyish-white tipped with red, and the legs and feet are yellow. There is a black throat patch and a band of cobalt-blue encircles the head completely.

Separate extracts of the head and various body contour feathers were made in 95 per cent alkaline methanol and in pyridine. Extraction was carried out in small batches over steam. Initial partition of alkaline-ethanol extracts with *n*-hexane or petrol ether partially resolved with crude ethanolic extracts into hypophasic and epiphasic layers. Each phase was washed three times with the opposite solvent, and the washings were pooled and added to the original extract. Because no interfacial salts were produced during partitioning, we assumed no pigments with carboxylic functional groups were present.

Pyridine extracts were either used directly for some spectral analysis or the pigments were transferred to a more polar solvent by dilution. There were no differences in the pigments produced by the two extraction procedures as measured by spectral tests or by co-chromatography. In many cases pyridine is more desirable as an extraction medium than alkaline ethanol as the latter produces artifacts (alkoxide salts) with certain pigments. Pigments that remained hypophasic in the ethanol:*n*-hexane partitioning system were forced into petrol ether or *n*-hexane by the method of Rothblat *et al* (1964).

Absorption spectra were recorded on a Cary Recording Spectrophotometer (Model 11). Partition coefficients were determined by the method of Petracek and Zechmeister (1956) and M_{50} values according to Krinsky (1963). Determination of the nature of various functional groups was by the reduction of keto groups with sodium borohydrate (Krinsky and Goldsmith, 1960) and conversion of epoxides with acid chloroform (Karrer and Jucker, 1950). Acetylation with acetic anhydride was used to test for hydroxy groups (Bamji and Krinsky, 1966) on both suspected feather pigments (xanthophyll) and on known xanthophyll. The xanthophyll epoxide structure was further identified by reaction with acetyl chloride and reaction with strong acid.

Crude extracts of pigments were separated by both column and thin-layer chromatography (TLC). Alumina was used exclusively in the chromatographic columns. The columns were poured dry, under slight pressure, on to a pad of glass wool. Columns were developed with either benzene and petrol ether or benzene and ethyl acetate and the bands removed mechanically (Fox, 1953). The individual pigments were then eluted with methanol, filtered, and forced into petrol ether by the addition of water. In nearly all cases this treatment produced completely epiphasic fractions.

The transfer of strongly hypophasic pigments was aided by the addition of a mixture of equal parts benzene and petrol ether. Columns were also used to prepare known carotenoid pigments: taraxanthin and violaxanthin from the dandelion (*Taraxacum officinale*), lutein from corn (*Zea mays*), and canthaxanthin from feathers of the male Scarlet Tanager (*Piranga olivacea*). These pigments were used as reference compounds.

Thin-layer chromatography (Stahl, 1965) was carried out on oven-dried preprepared silica gel plates (Eastman Kodak Chromatograms). The plates were developed with benzene-ethyl acetate (2:1). A benzene-acetone (98:2) system produced comparable separation, but the material oxidized more rapidly after separation than in the benzene-ethyl acetate system. For comparative purposes TLC was preferred to columns, as the resolving power was greater and the separation quicker. Furthermore, up to ten samples could be co-chromatographed simultaneously.

Whole mounts of feathers from various parts of the head and body of *P. gouldiae* were made with Canada balsam. In addition, mounts of carotenoid-containing feathers from various body areas were made from individuals of the following species:

Piranga olivacea
Piranga ludovicianus
Richmondena cardinalis
Carpodacus purpureus
Pheucticus ludovicianus
Acanthis flammea
Setophaga ruticilla
Tyrannus tyrannus
Dendroica fusca
Dendrocopos villosus
Colaptes auratus
Regulus satrapa
Melittophagus gularis
Lybius torquatus
Pipra aureola
Ajaia ajaja
Phoenicopterus roseus

RESULTS

Feather structure.—Melanin-containing feathers from the face of the black-headed birds were characterized by numerous relatively long barbs which diverged from a short rachis. Each barb was covered with short, thick barbules which contained heavy deposits of melanin (Figure 1A). (As in previous communications from this laboratory we prefer a restrictive usage of the term barb. On a feather, the barb is the primary branch of the rachis which normally supports the barbules. This usage is synonymous with the term ramus.)

The carotenoid-containing feathers of the face also possessed only a short rachis. However, unlike the melanin-containing feathers, the barbs were flattened and typically lacked barbules in areas of carotenoid deposition. A few fine barbules were present in the medial, unpigmented areas of the barbs (Figure 1B, 1C).

A similar relationship between structural elements and pigmentation was noted in the more complex feathers of the neck of *P. gouldiae*. The longer barbs on these feathers had three different areas of coloration. The proximal portion of each barb had numerous barbules and the entire area was heavily pigmented with melanin granules. The middle portion of the feather consisted entirely of sections of the barbs that lacked barbules and

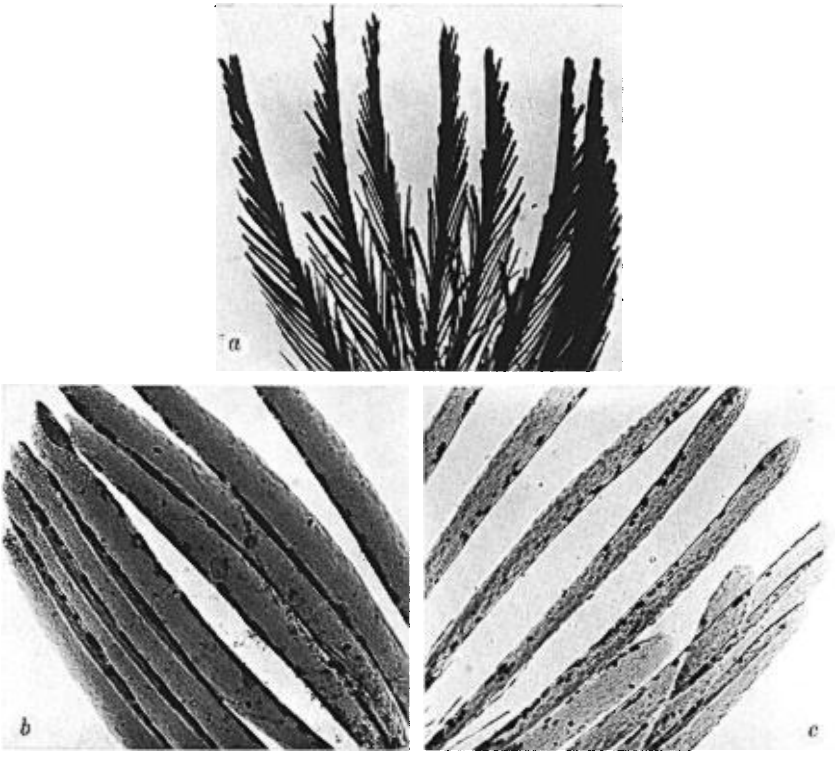

Figure 1. Structure of feathers from the face of the three color phases of *P. gouldiae*. A, black face, distal end of rachis and barbs with barbules; B and C, barbs of red and orange face respectively. Note lack of barbules on barbs in these two color phases. Actual length of feathers was 2 mm.

contained carotenoid pigment; this area was red in reflected light. In the most distal portions of the feather the carotenoid pigment was absent from the barb and the feather appeared blue in reflected light but was black under transmitted light. This was an area characterized by structural coloration. Feathers such as this demonstrate the precise control over the regulation of pigment and structure necessary to produce a highly patterned plumage.

Pigmentation.—The pigments isolated from the lilac breast feathers, the yellow feathers of the abdomen, and the green feathers of the back and mantle were yellow in solution and appeared yellow microscopically. The spectrum of the pigment was typical of hydroxy-containing carotenoids. Spectral data, partition coefficients, and chromatographic data are summarized in Table 1.

From the evidence presented here we conclude that the pigment present

TABLE 1

SUMMARY OF SPECTRAL AND CHEMICAL DATA ON CAROTENOIDS FROM THE FEATHERS OF THE GOULDIAN FINCH[1]

	R_f		*Partition coefficient*	*Spectra*	*Reference*
A. *Yellow abdomen*	81.2*	71.2	13:87	Ethanol: 471, 444, 425	This study
Green back		69.8	11:89	475, 445, 419	This study
Lilac breast		66.5	–	–	This study
Xanthophyll		70.9	12.5:87.5	474, 445, 423	Prepared Standard
Xanthophyll				476, 446, 420	Karrer and Jucker, 1950
Xanthophyll			12:88		Petracek and Zechmeister, 1956
B. *Red face*	86.3		48:52	Ethanol: 464 pyridine: 480	This study
Canthaxanthin	88.0		47:53	464 483	Prepared Standard
Canthaxanthin				Hexane: 466 benzene: 480	Völker, 1961; Lee, 1966
Astaxanthin (as Astacene)	78.2		25:75		Prepared Standard
Astaxanthin				Hexane: 469 pyridine: 500 petrol ether: 470	Karrer and Jucker, 1950; Fox, *et al.*, 1965; Lee, 1966
C. *Orange face*	83.2	68.6*		Ethanol: 472, 448, 430 pyridine: 490, 460, 438	This study
Xanthophyll epoxide				473, 455, shoulder	This study
Xanthophyll epoxide				473, 445	Karrer and Jucker, 1950
Orange head-acetate		74.5			This study
Violaxanthin	94.4			471, 441, 418	This study
Canary xanthophyll				472, 443, 418	Karrer and Jucker, 1950

[1] Xanthophyll (lutein) from *Zea mays* was used as a standard on all thin-layer plates. Partition coefficients are in *n*-hexane: 95 per cent methanol and were read at the absorption peak. Starred Rf's (= distance migrated by individual spot relative to distance of solvent front from starting point) indicate minor fractions.

in the yellow, green, and lilac feathers of *P. gouldiae* is lutein, 3-3′-dihydroxy-α-carotene. This pigment has been referred to as xanthophyll, a name now used as a generic term for all hydroxy-containing carotenoids. The generic term xanthophyll is equivalent to the term phytoxanthin. The identity of this pigment was confirmed by co-chromatography with lutein prepared from *Zea mays* (Karrer and Jucker, 1950).

Several features of individual pigment extracts are noteworthy. The yellow abdominal feathers contained a minor band on TLC that had the same mobility as the major band of the orange face extract. The reverse condition was found in the yellow face extract, which had a minor band with a mobility similar to that of the yellow abdominal feathers. No spectral data were obtained for the pigment extracted from the lilac breast feathers as the pigment was present in exceedingly low concentration. The pigments in the face feathers of the black-headed phase are presumably melanins. No carotenoids were obtained from these feathers even after extensive extraction.

The absorption spectra, relative mobility, and partition coefficient of the pigment extracted from the facial feathers of the red-headed form identify this compound as canthaxanthin, 4-4′-diketo-β-carotene (Table 1B). This was confirmed by co-chromatography with canthaxanthin prepared from the Scarlet Tanager. Spectral, chromatographic, and partition data exclude the possibility that the pigment of the red head was astaxanthin as was reported earlier (Völker, 1964).

Reduction of this pigment with sodium borohydride changed the spectrum from the typical single peaked diketone curve, to the three peaked curve typical of the dihydroxy-carotenoids. The spectrum of reduced canthaxanthin as well as other data identify this compound as isozeaxanthin, 4-4′-dihydroxy-β-carotene.

The absorption spectrum of the pigment from the facial feathers of the orange-headed *P. gouldiae* was similar to that of lutein (Table 1). On TLC two bands were present in the crude extract, a slow major one and a faster minor one. The minor band had a mobility similar to the major band in yellow body feathers and was therefore identified as lutein. The major band was less polar than lutein, but had a similar spectrum. Reaction of this pigment with either acetyl chloride or acetic anhydride in pyridine produced a more polar reddish pigment, presumably an ester. Reaction of the molecule with strong acid (HCl) changed the spectrum. Similar treatment had no effect on the spectrum of lutein, but did change its mobility. The chromatographic evidence eliminates the possibility of the presence of significant quantities of lutein in the orange-face form. The chemical evidence suggests that the pigment is lutein (xanthophyll) epoxide. Lutein epoxide is known to have a spectrum similar to xantho-

phyll but to be extremely unstable towards strong acid, the pigment being converted to isometric furanoid oxides (Karrer and Jucker, 1950).

On the basis of the evidence presented here, the pigment in the orange face could be either lutein epoxide, 3-3′-dihydroxy-5,6-epoxide-α-carotene or violaxanthin, 3-3′-dihydroxy-5,6-5′,6′-diepoxy-β-carotene. Co-chromatography with violaxanthin isolated from dandelions (*Taraxacum officinale*) eliminated the latter possibility. On our present evidence we identify the pigment as lutein epoxide. Some possibility exists that the pigments known as canary xanthophyll and lutein epoxide are the same molecule; in the absence of further information on the structure of the former, we agree with Karrer and Jucker and prefer to consider them not the same at this time.

DISCUSSION

The display of integumentary color, so conspicuously developed in birds, has numerous ecological and behavioral functions. The mechanical basis for such displays lies in the structure of the feathers, the distribution of the pigmented feathers over the surface of the body, and the motor patterns involved in a given display. The chemical basis lies in the particular pigments found in the feather and, in some cases, in the structural elements of the feather itself (e.g. "schemochromes"). We are concerned here with patterns on the body surface produced by differential production and deposition of carotenoids and colors produced by these pigments without structural interference.

As the result of various metabolic, chemical, genetic, and nutritional considerations, not all birds deposit carotenoid pigments in their feathers. Some orders lack carotenoids completely (for recent partial review see Völker, 1961). In those groups that produce carotenoid pigments and deposit them in their feathers, pigments may be distributed to produce either a monochromatic coloration or a well-defined multicolored pattern. In monochromatic forms such as the Threskiornithidae and Phoenicopteridae (Fox, 1962; Fox and Hopkins, 1966*a*, *b*) or certain cotingas (i.e. *Xipholena*, Völker, 1952; Brush, in press) feathers may contain either rather complex mixtures of chemically closely related pigments or almost pure deposits of a single pigment as in the North American tanagers (Brush, 1967). One extreme form of the latter case is tipped feathers of the Cedar Waxwing where carotenoids are deposited in a specialized structure on the tips of the secondaries (Brush and Allen, 1963). In multicolored patterned forms such as *P. gouldiae*, the patches of color appear to contain only a single pigment. The data now available suggest that carotenoids are not common in phylogenetically primitive forms or young birds (Desselberger, 1930) and that phylogenetically advanced birds are genetically capable

of producing both complex distributional patterns as well as monochromatic deposits of single pigments or pigment mixtures.

Because of the presumably greater metabolic expense involved in the production of carotenoids as compared to melanins, one might expect the evolution of efficient structural elements for display in feathers that contain carotenoids. Frank (1939), Rawles (1960), and others mention such a structure, but it has not been described in detail in a large number of species. The facial feathers of the Gouldian Finch demonstrate this structure clearly. The carotenoid-containing feathers have a short rachis, lack barbules, and have expanded and flattened barbs. All barbs are flattened in the same plane, thus exposing the greatest possible area towards the viewer. This condition is well developed in the Gouldian Finch where highly modified carotenoid-containing feathers are present on the face. It is not present in large monochromatic species where no noticeable feather modification for carotenoid displays have been described. The pigment quality in the small, structurally modified feathers is consistently more intense than in the larger unmodified contour feathers. Highly modified carotenoid-containing feathers are present on the head, neck, or throat of other species including: *Lybius torquatus, Melittophagus gularis, Dendrocopos villosus, Tyrannus tyrannus, Acanthis flammea,* and *Carpodacus purpureus.* An intermediate arrangement, where larger areas of color are displayed and the feather modification is not as extreme, is found in *Piranga olivacea* (heads of male in spring), *Richmondena cardinalis, Pheucticus ludovicianus, Piranga olivacea* (male, in fall plumage and female), *Piranga ludovicianus* (male body feather, but not the head), and *Dendroica fusca.*

On the basis of these expanded and rather consistent observations, we suggest that carotenoid-containing feathers, where they occur in specific patterns or in relatively small areas of intense coloration on monochromatic birds, are frequently modified in a manner that produces maximum exposure of the pigment. This modification consists of the reduction of potentially interfering pigments, especially melanins, the elimination of barbules, and the flattening of the barb to increase the exposed surface area (Figure 1). In many cases the barbs are longer than the feather shaft. As feather size, body size, or the area of pigmentation increases, these structural adaptations may be reduced or lost completely. We suggest further that not only are structure and carotenoid pigmentation closely related, but that the structure persists even in the presence of a modified carotenoid metabolic pathway. Precisely such a condition was found in the orange-face phase of *P. gouldiae.* It now appears that this combination of feather structure and carotenoid pigmentation has evolved in numerous

groups quite independently of taxonomic affinities. The display of pigment must be considered one of several primary functions of feathers.

The pathways for the production of pigment and structure of the facial feathers of the Gouldian Finch represent at least two separate control systems. No carotenoid pigments were found in the facial feathers of the black-headed form, which indicates that this color phase lacks completely the ability to produce the carotenoid facial color. This is not simply a matter of the melanins masking the carotenoids, as none of the carotenoids typical of the red-faced individuals was found. By implication the black-faced animals lacked the ability to metabolize β-carotenes or preferentially excrete them. The body feathers in black-faced birds, as was the case with the body feathers of the other color phases, deposit α-carotenes exclusively. We have no information on the nature of the pigment responsible for bill coloration. Feather structure in the black-face phase showed no significant structural modification.

Further evidence for the independence of the genes for feather structure and pigmentation comes from information on mutations. Immelmann (1965) described a lutino mutant in the Gouldian Finch where all body feathers are yellow and the face red. This condition presumably was caused by the absence of the melanin gene (i.e. a melanic albino) which exposed the yellow pigmentation of the feathers of the back and wings. Because carotenoids were not involved, the face remained red. A blue-backed mutant was described also, probably the result of the deletion of the carotenoid pathway which exposed structural coloration in the blue areas. Immelmann (*loc. cit.*; pers. comm.) gives no information on the color of the head or abdomen, and Tordoff informs us quite informally that he believes the under parts were white. This would support our suggestion that the mutation involved the deletion of the carotenoid pathway.

In the red-faced forms of *P. gouldiae* the feather structure of the carotenoid-containing feathers on the head was changed markedly, melanins were not present in any significant amount and the animal was capable of depositing at least two carotenoid types. This implies the presence of one genetic system that controls feather form and a second for the control of feather pigment content. The differences in the chemical structure of the pigment in the facial feathers (β-carotenes) and the body feathers (α-carotenes) suggest also the presence of at least two pathways for the metabolism of carotenoid pigments from different precursors. On the assumption that it is chemically both easier in a steric (i.e. spacial or conformation) sense and more efficient in an energetic sense to modify functional groups than to change the structure of the carotene backbone, the appearance of the β-carotene in the red facial feathers is unexpected.

One might have predicted the presence of the diketo derivative of lutein (e.g., 3-3′-diketo-α-carotene) rather than the diketo-β-carotene, canthaxanthin. Diketo-α-carotenes have not been reported from birds, and the 4-4′-diketo form is not stable chemically. We conclude, therefore, that a second synthetic pathway for carotenoids has developed in this phase. Lutein is deposited in the body feathers and canthaxanthin, presumably derived from β-carotene or isozeaxanthin, in the facial feathers.

In general, the carotenoids in animals, especially the carotenes and xanthophylls, are believed to be obtained either directly or indirectly from the food. Compelling evidence for this position in regards to birds comes from Völker's (1955) feeding experiments on canaries. However, pigments such as astaxanthin, rhodoxanthin, and canthaxanthin are considered specific animal metabolic derivatives of these precursors (Völker, 1963; Brush, 1967). Lee (1966) suggested a possible metabolic pathway linking β-carotene to canthaxanthin. His data indicated that the β-carotenes were metabolized independently, but simultaneously with lutein. The data of Fox and Hopkins (1966*a*, *b*) also suggest relatively independent pathways for α- and β-carotene and their derivatives in flamingos. A simple metabolic relationship between isozeaxanthin and canthaxanthin, two β-carotene derivatives, is responsible for the seasonal change of color in plumages of the Scarlet Tanager (Brush, 1967). Further evidence for independent pathways of the metabolism of α- and β-carotenes comes from the isotopic labeling studies of Williams *et al* (1967). These studies show that the α- and β-ionone rings in cyclic carotenoids are formed independently and not from isomerization of one form into another. The most likely mechanism is by the elimination of different protons from the same carbonium ion intermediates. The evidence presented in the present paper indicates the existence of a similar metabolic situation in the Gouldian Finch. The pigment of the yellow body feathers (α-carotene) and the red face feathers (β-carotene) are metabolized independently while the orange face feather pigment (α-carotene) is derived from the lutein of the body feathers. We suggest, therefore, that although there does not seem to be any marked taxonomic specificity or phylogenetic sequence to the appearance of specific carotenoids, the biochemistry of these molecules is capable of evolutionary change (Zuckerkandl and Pauling, 1965). The mechanism for production of individual carotenoids is specific and limited in part by the internal structure and functional groups in a particular sequence.

Völker (1964) correctly identified the pigment in the yellow body feathers of *P. gouldiae* as lutein, but he suggested that because of its genetic makeup the orange-faced phase was not able to transform lutein into the oxidized state of the red-faced pigment (which, incidently, he incorrectly identified as astaxanthin). Völker claimed that this incapacity

was the result of a gene deletion, and lutein was deposited unchanged producing the orange facial color. Our findings disagree with this interpretation. If, in fact, the orange face does not represent the specific production of color but instead is a "dump" for the end products of carotenoid metabolism, then one would expect to find an admixture of unrelated pigments and pigment products present. However, this was not the case. The orange-face mutant contained a unique pigment. Völker interpreted the orange-face genotype as a mutant which lacked the enzyme to produce a red pigment, a gene deletion. The genetic evidence (Murray, 1963) would require not only a deletion mutation (or repressor activity) but the presence of a second gene as well. The orange phase is produced only when the bird is homozygous for this autosomal recessive. The gene that produces the orange face is genetically independent from the production of red or black. We submit that the orange face is controlled by this second gene for carotenoid coloration and that the pigment is specifically derived from the lutein of the body feathers and not a separate precursor as is the case in the red-faced genotype. The fact that the gene that produced the orange face is not sex-linked, but autosomal and therefore on a different chromosome, argues against the orange face being due to a simple mutation of the red gene. We assume that the red, sex-linked gene is inactivated either by deletion, repression or mutation and the product of the second gene is capable of converting the yellow body α-carotene to the derived xanthophyll of the orange face. Further evidence for the condition described here comes from the bill coloration in Southern's "type C" genotype. In this case the recessive black genotype produces an orange beak, where red appears in all other cases.

The pigment present in the orange-faced phase of *P. gouldiae* is lutein epoxide an oxidative derivative of lutein (Figure 2). Lutein is present but only in an amount barely detectable on TLC. No other carotenoids were present in the extracts from the yellow-faced form. Because of the similarities in the spectra of lutein and its epoxide, Völker's conclusion was understandable, as it is difficult to differentiate these pigments spectrophotometrically, but partition coefficients and co-chromatography show that the pigment in the orange face is not lutein and suggest strongly that it is lutein epoxide.

If the arguments presented above for the nature of the control of the red and black faces hold, then the orange face may be explained easily. The feather structure is the same as in the red face (Figure 1), a structure typical of many small carotenoid-containing feathers. The gene for feather structure is unmodified. The pigment deposited is a natural product of the oxidation of the yellow pigment in the body feathers. A chemical change such as that required to produce the epoxide could be accomplished

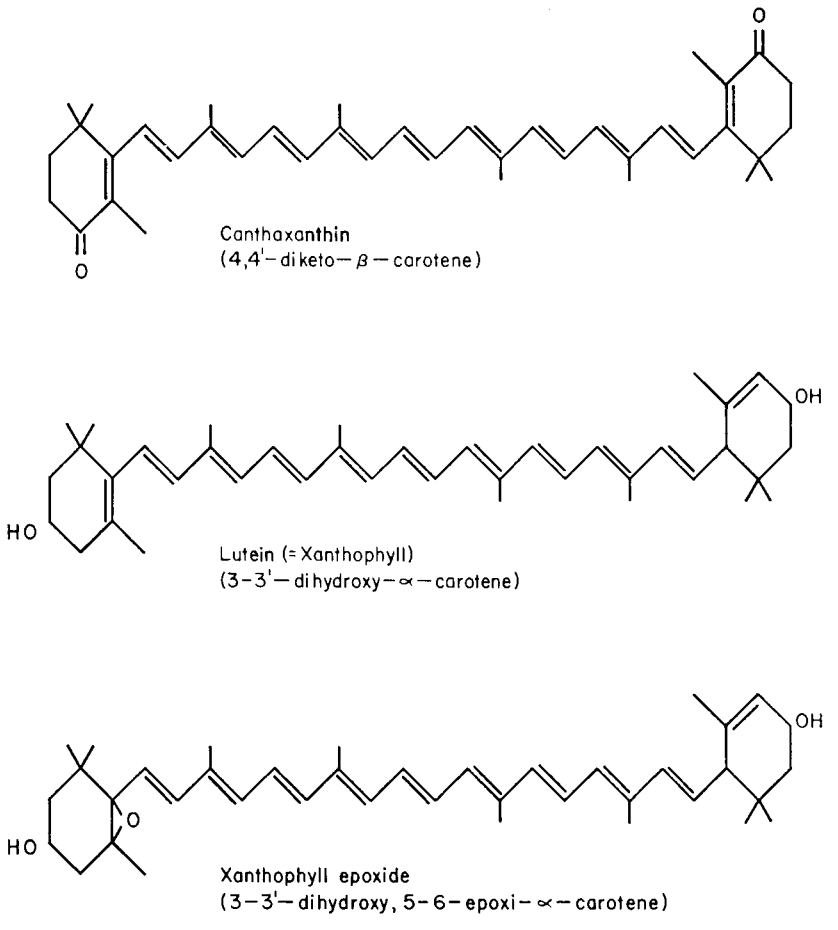

Figure 2. Structural formulae of cartenoid pigments partly responsible for coloration in the Gouldian Finch. Evidence presented here indicates separate pathways for the α- and β-carotene backbones. The close structural relationship between lutein and lutein epoxide is important in understanding the origin of this color variant.

by a single enzyme. This enzyme is produced by a gene involved with color production and display but, unlike that for the red face, not sex-linked and expressed phenotypically only as a homozygous recessive. The apparent location of this gene on an autosomal rather than a sex chromosome suggests that it is a separate gene and not the direct result of the mutation of the red face gene, a suggestion supported by genetic analysis. Our statement that the epoxide is a natural product of lutein is supported further by co-chromatography comparisons. The yellow body feathers of

orange-faced individuals carried a trace of the facial epoxide and the facial feather extract a trace of body pigment. No lutein epoxide or canthaxanthin was found in the yellow body feathers of the red- or black-faced birds. These data indicate that the two pigment types are completely separated in the feathers of each area. The mechanism responsible for such selectivity is still unknown.

On the evidence presented here we suggest that the facial coloration of *P. gouldiae* is under the control of at least two sets of genes. One set is responsible for the structures observed and the second for the metabolic production of the colors. Evidence from the chemical studies indicates that the pathways for pigment production may be quite complex. A third area of potential genetic control is the production of the patterns themselves. The mechanism of the precise localization of the carotenoid pigments, melanins, and structural colors in the plumage is completely unknown. However, two mechanisms regarding the differential deposition of carotenoids may be suggested. First that the cells of the follicle are capable of selectively assimilating and metabolizing specific carotenoid precursors which subsequently appear in the feathers. That is, the specific pigment end products are produced in a central area, perhaps the liver, and then selectively and unerringly removed from the circulation and subsequently deposited in the developing feather.

An alternative arrangement is that all precursors are assimilated by the follicular cells and that certain precursors are then modified chemically into the final pigment product. Other, unusable precursors are excreted. In this case the specificity resides in the enzymes of the cells of the follicle or the feather germ rather than in its selectivity. Our present knowledge of the biochemistry and fractionation of carotenoids in vertebrates and the development of carotenoid-containing feathers does not allow a definitive answer to these questions.

SUMMARY

The three color phases of the Gouldian Finch, *Poephila gouldia*, were studied with regard to feather structure and pigmentation. The facial feathers of the carotenoid-containing variants have a highly modified structure presumably for the efficient display of the metabolically produced pigments. This structure, characterized by the absence of barbules and an expanded and flattened barb, was found to occur in many other species that have relatively small areas of intense plumage coloration.

The pigment in the body feathers of all color phases was identified as lutein, 3-3′-dihydroxy-α-carotene, the pigment in the red-headed form as canthaxanthin, 4-4′-diketo-β-carotene. The evidence currently available indicates that the metabolism of α- and β-carotenes is under separate genetic control. The feathers of the rare orange-faced variant contain lutein

epoxide, 3-3′-dihydroxy-5, 6-epoxide-α-carotene. The epoxide appears to be derived directly from lutein, but to be controlled by a different gene from those that produce the red and black phases. Color production and feather structure in this species are closely allied but appear to be under different genetic control.

Some aspects of the evolution of carotenoid metabolism in birds and the genetic and biochemical control necessary to produce a patterned and colored plumage are discussed.

ACKNOWLEDGMENTS

We thank H. B. Tordoff, University of Michigan Museum of Zoology, who generously donated materials and critically read the manuscript. Seifried was supported by an NSF Undergraduate Research Participation Grant. The work of Brush is supported by NSF grant GB4710.

LITERATURE CITED

BAMJI, M. S., AND N. I. KRINSKY. 1966. The carotenoid pigments of a radiation-resistant Micrococcus species. Biochim. Biophys. Acta, **115**: 276–284.

BRUSH, A. H. 1967. Pigmentation in the Scarlet Tanager, *Piranga olivacea*. Condor, **69**: 549–559.

BRUSH, A. H., AND K. ALLEN. 1963. Astaxanthin in the Cedar Waxwing. Science, **142**: 47–48.

BUTLER, A. W. 1902. On the changes of plumage and variations thereof in *Poephila gouldiae*. Ibis, **2** eighth series: 674–676.

DESSELBERGER, H. 1930. Ueber das Lipochrome der Vogelfeder. J. f. Orn. **78**: 328–376.

FOX, D. L. 1953. Animal biochromes and structural colours. Cambridge, England, Cambridge Univ. Press.

FOX, D. L. 1962. Metabolic fractionation, storage, and display of carotenoid pigments by flamingoes. Comp. Biochem. Physiol., **6**: 1–40.

FOX, D. L., AND T. S. HOPKINS. 1966*a*. Comparative metabolic fractionation of carotenoids in three flamingo species. Comp. Biochem. Physiol., **17**: 841–856.

FOX, D. L., AND T. S. HOPKINS. 1966*b*. β-Carotenoid fractionation in the Scarlet Ibis. Comp. Biochem. Physiol., **19**: 267–278.

FOX, D. L., T. S. HOPKINS, AND D. B. ZILVERSMIT. 1965. Blood carotenoids of the Roseate Spoonbill. Comp. Biochem. Physiol., **14**: 641–649.

FRANK, F. 1939. Die Färbung der Vogelfeder durch Pigment und Struktur. J. f. Orn. **87**: 426–524.

IMMELMANN, K. 1965. Australian finches. Sydney, Australia, Angus and Robertson.

KARRER, P., AND E. JUCKER. 1950. Carotenoids. New York, Elsevier Publishing Co.

KRINSKY, N. I. 1963. Relationship between partition coefficients of carotenoids and their functional groups. Anal. Biochem., **6**: 293–302.

KRINSKY, N. I., AND T. H. GOLDSMITH. 1960. The carotenoids of the flagellated alga, *Euglena gracilis*. Arch. Biochem. Biophys., **91**: 271–279.

LEE, W. L. 1966. Pigmentation of the marine isopod *Idothea montereyensis*. Comp. Biochem. Physiol., **18**: 17–36.

MURRAY, R. 1963. The genetics of the yellow-masked Gouldian Finch. Avicult. Mag., **69**: 108–113.

PETRACEK, F. J., AND L. ZECHMEISTER. 1956. Determination of partition coefficients of carotenoids as a tool in pigment analysis. Anal. Chem., **28**: 1484–1485.

RAWLES, M. E. 1960. The integumentary system. *In* Biology and comparative physiology of birds, vol. 1 (A. J. Marshall, ed.). New York, Academic Press.

ROTHBLAT, G. H., D. S. ELLIS, AND D. KRITCHEVSKY. 1964. The carotenoid pigments of *Micrococcus lysodeikticus*. Biochim. Biophys. Acta, **84**: 340–347.

SOUTHERN, H. N. 1946. The inheritance of head colour in the Gouldian Finch. Avicult. Mag., **52**: 126–131.

STAHL, E. 1965. Thin-layer chromatography. A laboratory handbook. New York, Springer-Verlag Co.

VÖLKER, O. 1952. Die Lipochrome in den Federn der Cotingiden. J. f. Orn., **93**: 122–129.

VÖLKER, O. 1955. Die experimentelle Rotfärbung der Vogelfeder mit Rhodoxanthin, dem Arillus—Farbstoff der Eibe (*Taxus baccata*). J. f. Orn., **96**: 54–57.

VÖLKER, O. 1961. Canthaxanthin in Vogelfedern. Naturwissenschaften **17**: 581–582.

VÖLKER, O. 1962. Experimentelle Untersuchungen zur Frage der Entstehung roter Lipochrome in Vogelfedern. J. f. Orn., **103**: 276–286.

VÖLKER, O. 1963. Die Bedeutung der Carotinoide für das tierische Farbkleid, erläutert an einigen Beispielen. Carotine und Carotinoide, **9**: 282–291.

VÖLKER, O. 1964. Die gelben Mutanten des Rotbauchwürgers (*Laniarius atrococcineus*) und der Gouldamadine (*Chloebia gouldiae*) in biochemischer Sicht. J. f. Orn., **105**: 186–189.

WILLIAMS, R. J. H., G. BRITTON, AND T. W. GOODWIN. 1967. The biosynthesis of cyclic carotenes. Biochem. J., **105**: 99–105.

ZUCKERKANDL, E., AND L. PAULING. 1965. Molecules as documents of evolutionary history. J. Theoret. Biol., **8**: 357–366.

Biological Sciences Group, University of Connecticut, Storrs, Connecticut 06268.