Sexual dichromatism in birds independent of diet, parasites and androgens

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SUMMARY

Sexual selection may explain why male animals are typically more colourful than females. Females may choose brightly coloured males for mating because colour is a reliable signal of a male's genetic resistance to parasites, or that he can bear the cost of the immunosuppressant effects of androgens. Bright yellows, oranges and reds are the product of carotenoid pigments, which are known to have significant health benefits. Therefore, bright colours may be indicative of a bird's quality because it shows access to a superior diet or superior foraging ability. We maintained populations of American kestrels and loggerhead shrikes in captivity that were largely free of parasites and fed a uniform diet. Male kestrels were more brightly coloured than females in the colour of their ceres, lores and legs, and there were pronounced age- and gender-specific patterns to concentrations of carotenoids in their plasma. Even though shrikes do not show any carotenoid-based colouration, the sexes had pronounced differences in plasma carotenoids. Carotenoids in kestrels were unrelated to androgen levels, but the correlation between carotenoids and plasma proteins suggest colour may be a condition-dependent trait. These results suggest that neglected physiological processes may regulate carotenoids, and hence some colour variation need not be explained by parasites, androgens or diet.

1. INTRODUCTION

Why males are more brightly coloured than females in many animals, particularly birds, has been the subject of considerable debate (Cronin 1991). Charles Darwin's (1871) theory of sexual selection, and more specifically his mechanism of female choice, has received considerable attention in recent times (Andersson 1994). While there is general agreement that brightly coloured males can have an advantage in obtaining mates, how females benefit by such choice is more contentious (Johnstone 1995). Hamilton & Zuk (1982) stimulated considerable interest when they proposed that bright colouration was a reliable signal of a male's genetic resistance to parasites. Discriminating females may thus benefit by picking a high-quality mate. An extension of the Hamilton-Zuk hypothesis is the immunocompetence handicap theory (Folstad & Kartner 1992). Many secondary sex traits perceived to be the product of sexual selection are under hormonal control (Owens & Short 1995). Although bright colour or other ornaments may confer an advantage in acquiring mates, hormone production is not without potential fitness costs. Androgens suppress the immune system (see review by Zuk 1994). Only high-quality males may be able to pay the price of androgen levels required to maintain bright colouration or other secondary sex characters (Folstad & Kartner 1992). Once again, colour has the potential to function as a signal of male condition to females that may lead to adaptive mate choice.

Unlike many other sexually selected traits such as elongated feathers or other ornaments, colour can have a special relation with diet. In particular, the bright reds, yellows and oranges so pervasive in the avian world, are the product of carotenoid pigments. These compounds can not be synthesized *de novo* in animals, and so ultimately the origin of such colours in birds is dietary (Brush 1981, 1990). Despite the considerable importance of carotenoids in maintaining captive birds (Brush 1981), and enhancing the marketability of poultry (Marusich & Bauernfeind 1981; Williams 1992), relatively little is known of carotenoid physiology or consideration given to the fact that these pigments are not inert (Brush 1990; Fletcher 1992; Hencken 1992). There is increasing awareness that carotenoids have significant health benefits; for example, they can be precursors of vitamins, boosters of the immune system, have anti-cancer effects, etc. (Bendich 1989; Lozano 1994; Britton 1995). The appreciation of the evolutionary significance of these intriguing compounds in wild birds is only a recent development (Brush 1990). By virtue of the relation between diet and colour, brightly coloured males are

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believed by some to have better access to quality foraging areas or have more skills at harvesting high-quality, carotenoid-rich foods (Endler 1980; Hill 1990). Colour therefore may be a condition-dependent trait that has the potential to allow females to discriminate among potential mates (Hill 1990, 1991).

Although it is well known that females choose males that possess certain traits (Andersson 1994), the confirmation of an association between those traits and male quality is far from convincing or free of methodological problems (Johnstone 1995). Designing appropriate experiments, or even comparative analyses, for testing the Hamilton-Zuk hypothesis has been difficult (Cronin 1991; Dufva & Allander 1995). Similarly, the validity of a link between colour, foraging and diet is controversial in that carotenoids may not be limited in the environment, and diet alone may not be responsible for variation in colour among males (Hudon 1994; Lozano 1994). There has generally been a failure to test concurrently the potential effects of diet, parasites and androgens on colour, while taking into consideration how the use and metabolic pathways of carotenoids may be further dependent on other gender- and age-specific processes.

Our approach has been to document sources of variation in colour, determine the potential physiological basis of that variation by quantifying carotenoids, and then explore possible causal links with parasites and androgens. Most studies of this kind naturally have focused on passerine birds with extreme sexual dichromatism of carotenoid-rich plumage (Slagsvold & Lifjeld 1985; Hill *et al.* 1994; Hill & Benkman 1995). Our use of two small, predatory species, the American kestrel (*Falco sparverius*) and the loggerhead shrike (*Lanius ludovicianus*), offers a unique perspective. Although phylogenetically unrelated, these two species are ecological parallels, and sympatric over large areas of North America; both are monogamous and prefer open habitats and feed on a variety of insects and small vertebrates (Bird 1988). Kestrels are sexually dichromatic in plumage (blue-greys and browns), but carotenoid-based colouration is limited to exposed integument on the face and legs. Shrikes are sexually monochromatic with no expression of any red, yellow or orange on feathers or skin. Our use of populations that have been maintained in captivity over generations provides us with an ideal opportunity to control some potential sources of variation in colour and carotenoids, while investigating others to a degree of magnitude and detail not possible in most studies. We suggest that patterns of colouration and plasma carotenoid levels are evidence for so far neglected causal factors responsible for sexual colouration in birds, and substantiate the possibility that bright colours are associated with the quality of an individual, and hence colour may function in adaptive mate choice.

2. METHODS

We did this study at the Avian Science and Conservation Centre of McGill University, Quebec, Canada, in April 1995; a time when the birds (captive and wild) were about to form pairs. A colony of kestrels has been maintained there since 1974 (Bird 1982). A total of 400 kestrels were housed communally in unheated, indoor pens of 25–35 individuals segregated by sex. All these birds were of known age from 1–13 years for males, and 1–9 years for females, but for most analyses we categorized birds as being 1, 2, 3 or 4+ years of age. The captive population of shrikes began in 1992 with the collection of nestlings from Saskatchewan, Canada. The colony consisted of 19 birds in April 1995, and each shrike was housed in its own cage in an unheated pen adjacent to the kestrels. Of the shrikes used in this study, four males and two females were hatched at the Centre in 1993, while two males and one female were part of the original collection from the wild. Gender was determined surgically.

The kestrels had been maintained exclusively on a diet of day-old cockerels for 3 years before this study, and with the exception of a few birds for short periods the diet had been cockerels since 1980. Cockerels are a carotenoid-rich diet; they had yellow skin and orange-yellow tarsi, and poultry are maintained on a carotenoid-supplemented feed (Marusich & Bauernfeind 1981). The diet of shrikes was also cockerels, but with a minor supplement of insects for breeding birds. Shrikes ate only cockerels for at least 8 months before our work.

We evaluated the colour of the unfeathered ceres, lores and tarsi of kestrels, rather than plumage. There are several advantages of using 'fleshy parts'. First, unlike the red-browns (melanins) of kestrel plumage, the bright yellow and orange of the integument are convincingly associated with carotenoids. Second, the colour of feathers is only indicative of a bird's physiology at the time those feathers were grown. Because birds generally do not store carotenoid pigments (Brush 1990), the colour of fleshy parts is more suggestive of recent physiological events, and hence a better indicator of the potential physical condition of birds (Johnson *et al.* 1993; Lozano 1994; Owens & Short 1995). Third, comparing the colour of feathers among individuals may be complicated by differential feather wear, fading, and timing of moult. Last, birds lack chromatophores and so are constrained in the possibility of colour change (Brush 1990). The colour of feathers, on the other hand, can vary because of structural properties (Brush 1990). Therefore, we scored the colour of 121 male and 82 female kestrels by comparing the tarsi, ceres and lores to a six-option colour chart derived from paint samples. We considered birds to be increasing in brightness as the amount of orange increased. As 6 = pale yellow, 1 = red-orange, high scores are 'dull' and low scores are 'bright'. We derived a total colour score per individual by summing the scores of the three body parts.

From the same kestrels, we extracted 0.45 ml of blood from the jugular or brachial veins. We divided the plasma into two microcentrifuge tubes and froze them. To investigate the physiological basis of colour we measured plasma concentrations of carotenoids in one of these samples. Blood is the necessary intermediate between diet and deposition of carotenoids in dermal parts, and by quantifying pigments we avoid potential human limitations and biases of colour perception. Serum pigments are a good correlate of flesh colour in poultry (Fletcher 1992). To quantify carotenoids we diluted 0.1 ml of plasma with acetone (1:10), and precipitated the flocculant protein by centrifuging the sample at $1500 \times g$ for 10 min. We examined the supernatant in a Beckman Du-70 spectrophotometer and determined the optical density of the carotenoid peak at 476 nm. We estimated concentration using a standard curve for lutein (alpha-carotene-3, 3′-diol, Sigma Co.).

Plasma androgen levels were measure by radioimmunoassay (RIA) (Van Der Kraak *et al.* 1984). To eliminate possible interference from plasma steroid binding proteins, androgen measurement was done on reconstituted organic

(diethyl ether) extracts of the plasma samples; plasma androgen extraction efficiency was estimated to be consistently greater than 90%. Each extract was measured in duplicate at two dilutions (1:2 and 1:4 v/v relative to the original plasma sample); the intra- and inter-assay coefficients of variation were less than 5%. Serial dilution of sample extracts produced an inhibition curve parallel to the testosterone standard curve.

The RIA used an anti-testosterone serum purchased from Sigma Chemicals, ^{3}H-testosterone purchased from Amersham, and purified testosterone purchased from ICN Chemicals as the standard. The hormonal specificity of the anti-testosterone serum was determined from information provided by the supplier (Sigma). In addition to cross-reacting with testosterone (100%), the antiserum also displays a significant (23%) cross-reaction with 5α-dihydrotestosterone. 5α-dihydrotestosterone has been found in substantial amounts in birds (Wingfield & Farner 1980). As the RIA used in the present study likely detects both testosterone and 5α-dihydrotestosterone, and as 5α-dihydrotestosterone is biologically active, the RIA results are best represented as androgen levels rather than testosterone (Rehder *et al.* 1988).

The minimum plasma androgen level in the kestrel samples that could be detected with this RIA was 120 pg ml^{-1}. Some samples contained androgen levels below the detection limit, so for statistical purposes they were assigned a value equivalent to the detection limit.

To put our findings into perspective of the physical condition or 'health' of the kestrels, we evaluated total plasma proteins (Snyder *et al.* 1981; Stoskopf *et al.* 1983; De le Court *et al.* 1995) and blood parasites. A drop of plasma was read on a hand-held refractometer (Model 10400A, American Optical Corp.) to estimate protein concentration. In a subsample of kestrels, we prepared whole-blood smears for parasite analysis by G. F. Bennett (see Bennett *et al.* 1995).

We bled six male and three female shrikes. Although the full spectrum of blood analyses was done on kestrels, only carotenoids were quantified for shrikes.

3. RESULTS

The colour of the ceres, lores and tarsi of captive kestrels varied from a dull yellow to a bright red-orange, comparable to wild birds in Saskatchewan (G. R. Bortolotti, personal observation) and Quebec (J. J. Negro, personal observation). Consistent with the general avian pattern, male kestrels were more brightly coloured than females; the total colour scores of the sexes were significantly different within each age category (Mann-Whitney U tests, all $p < 0.009$) and all ages combined ($U = 1713.5$, $p < 0.0001$, see figure 1). The variation in colour among individuals within each sex appears, as expected, to be associated with the quantity of carotenoids. There was a significant correlation between total colour score and carotenoids for both males ($r = -0.279$, d.f. $= 118$, $p = 0.002$) and females ($r = -0.314$, d.f. $= 78$, $p = 0.005$, same results when age was controlled for in partial correlations). The difference in colour between the sexes appears to be associated with quantity of carotenoids, rather than a physiological effect of how external colour is expressed in males versus females. We compared the carotenoid concentration of males and females with a total colour score of ten (the modal value for both sexes, see figure 1), and found no difference (males: $x = 20.2$, s.e. $= 1.09$, $n = 73$; females: $x = 20.1$, s.e. $= 1.02$, $n = 28$, ANOVA $F_{1,99} = 0.001$, $p = 0.97$). Therefore, the more carotenoids in the plasma, the more brightly coloured the bird (see figure 2). Because carotenoid levels were objectively determined, and more relevant to physiological processes, we restrict further analyses to carotenoids rather than colour.

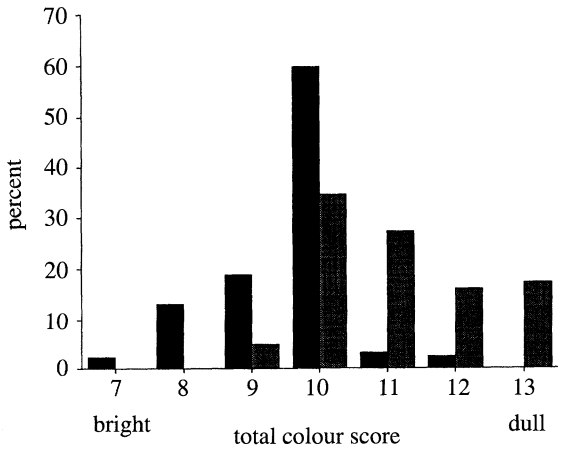

Figure 1. Frequency distribution of the total colour scores of male ($n = 121$; shaded) and female ($n = 82$; unshaded) kestrels.

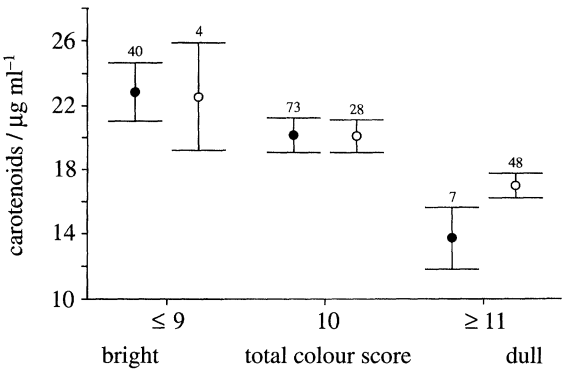

Figure 2. Plasma carotenoid concentrations of male (closed circles) and female (open circles) kestrels with colour scores of ⩽ 9 (bright), 10 and ⩾ 11 (dull). Birds at the extremes were combined because there are few bright females and few dull males. Points are mean values, bars are standard errors, and numbers above bars are sample sizes.

Consistent with our analysis of colour, there were gender differences in plasma carotenoids of kestrels; a two-way ANOVA revealed significant sex ($F_{1,195} = 4.23$, $p = 0.041$) and age ($F_{3,195} = 3.45$, $p = 0.019$) effects (no interaction) (see figure 3). The hypothesis that male shrikes had significantly more plasma carotenoids than females was similarly supported ($U = 2$, $p = 0.035$, one-tailed, see figure 3).

Despite the fact that androgens are frequently linked to sexually dimorphic and age-related traits, they do not appear to be responsible for the observed variation in carotenoids (see figure 3). We could not detect any difference in androgen levels among age categories of males (ANOVA $F_{3,111} = 1.35$, $p = 0.26$). There was also no association between androgens and age in years ($r = 0.15$, d.f. $= 80$, $p = 0.18$) or between androgens and plasma carotenoids ($r = 0.046$, d.f. $= 114$, $p = 0.62$)

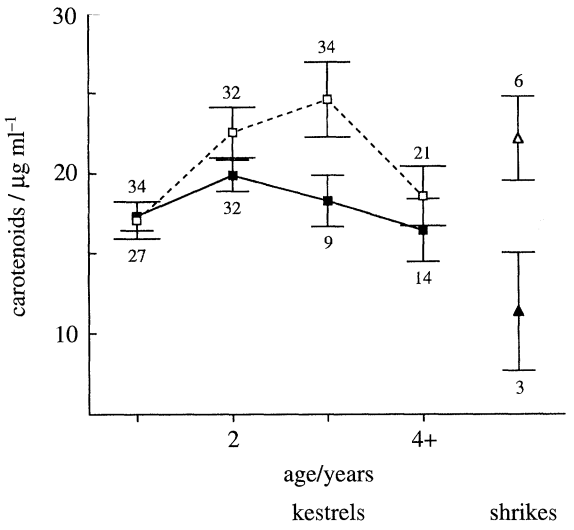

Figure 3. Plasma carotenoid concentrations of kestrels (squares) of four age categories, and all shrikes (triangles). Males are open symbols and females are solid symbols. Means are shown with standard errors, and sample sizes are above and below bars.

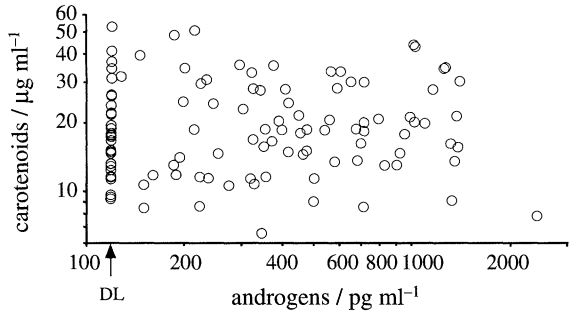

Figure 4. Scatterplot of concentrations of androgens and carotenoids in the plasma of male kestrels. DL denotes the detection limit (120 pg ml^{-1}) of the radioimmunoassay for androgens.

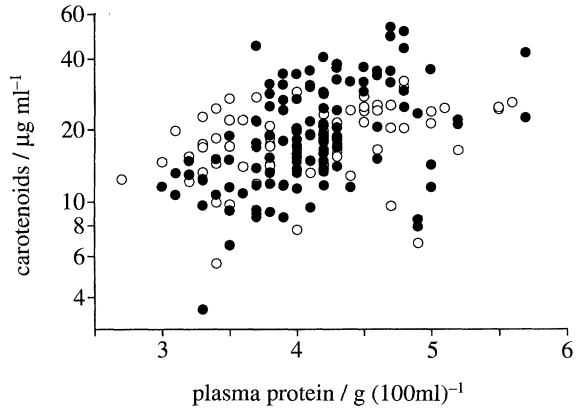

Figure 5. Relation between total plasma protein and plasma carotenoids of male (closed circles) and female (open circles) kestrels.

(see figure 4). The above analyses included samples at the detection limit; however, exclusion of these data did not alter any of the results.

Although parasites have been implicated as a causal agent in colour variation in birds, they are unlikely to be significant in this study. We could not detect any haematozoa in the 35 males (25 older than one year) and 42 females (37 older than one year) sampled. Similarly, in the repeated handling of all birds over the years we rarely encountered ectoparasites.

A two-way ANOVA revealed that plasma proteins, which may be indicative of overall physical condition, did not vary among age categories ($F_{3,191} = 0.91$, $p = 0.44$) or between the sexes ($F_{1,191} = 0.659$, $p = 0.42$). However, there were strong positive correlations between proteins and carotenoids for males ($r = 0.469$, d.f. = 115, $p < 0.0009$), females ($r = 0.360$, d.f. = 78, $p < 0.0009$) and the sexes combined ($r = 0.414$, d.f. = 195, $p < 0.0009$) (see figure 5).

4. DISCUSSION

Our understanding of physiological control of colour in birds is in its infancy (Brush 1990). Slagsvold & Lifjeld's (1985) study of carotenoid-based plumage in wild great tits (*Parus major*) is exceptional. They found colour varied within the nesting season, and between habitats, the sexes and ages (nestling versus adult). Furthermore, they were able to show experimentally how a large part of plumage variation was attributed to environmental rather than genetic factors. Hill *et al.* (1994) subjectively evaluated plasma hues in wild house finches (*Carpodacus mexicanus*), and confirmed that levels of circulating carotenoids correlated with feather brightness in males. The importance of diet in the proximate control of plumage colour of finches in captivity has been well established (Brush & Power 1976; Hill 1992, 1993). Hill *et al.* (1994) concluded that gender-specific differences in pigmentation of finches, as well as inter-male variation, occurred before uptake by the feather follicle rather than how carotenoids were selectively processed. We concur. However, Hill *et al.* (1994) concluded that colour variation was therefore likely caused by differences associated with the uptake of carotenoids, such as foraging behaviour, diet quality or parasites. Kestrels exhibited considerable colour variation while on a uniform diet. Whereas variation in access to carotenoids at some level is critical, it may only explain a portion of the variability in colour observed among individuals within a species.

Despite the fact that female house finches get brighter when fed carotenoids (Hill 1993), it is clear that there remains inherent physiological effects on colour associated with gender. Hormonal effects on house finch pigmentation have been documented (Tewary & Farner 1973). Hill *et al.*'s (1994) relation between plasma carotenoids and colour in finches was significant only for males. In kestrels, the sexes showed the same association between colour and carotenoids, and had the same colour for a given concentration of pigments, yet they were very different in absolute colour and concentration of carotenoids. For house finches (Hill 1993), zebra finches (Burley *et al.* 1992), great tits (Slagsvold & Lifjeld 1985) and kestrels, females are not as brightly coloured as males despite what would seem to be an abundance of dietary pigments.

Our results also question the degree to which diet alone explains intrasexual variation, particularly age effects. Hill *et al.* (1994) found that age differences in plumages also correlated with plasma carotenoids, and believed this supported their idea that colour was explained by access to carotenoids. However, our kestrels showed similar age variation on a uniform diet.

Both gender and age-specific variation in carotenoids of kestrels suggest physiological control mechanisms independent of diet that have not been identified. Although there is growing evidence that hormones may be involved in sexual colouration (Tewary & Farner 1973; Brush 1990; Johnson *et al.* 1993), there was no relation between androgen concentrations and either age or plasma carotenoids in kestrels. Our results do not support the immunocompetence handicap theory.

The correlation between plasma proteins and carotenoids in kestrels, suggests causal factors in some way related to the condition of the bird, independent of gender, age, and parasites. Although we cannot exclude the possibility that there were unidentified pathogens, the paucity of common parasites suggests that we can likely reject the Hamilton–Zuk hypothesis. Empirical data linking condition to colour are relatively few for wild (Hill 1991; Burley *et al.* 1992; Dufva & Allander 1995; Johnstone 1995), but not captive (Hudon 1994; but see Hill 1994) birds. Whereas the rationale for colouring poultry products has been consumer choice, this preference is founded on the tradition that pale meat is indicative of an unhealthy bird, in particular, one infected with coccidiosis (Williams 1992).

Alfred Russel Wallace foreshadowed much of the current thinking on adaptive mate choice (Cronin 1991). To Wallace, colour was merely a correlate of 'vigour', by which he implied health. A female should choose a mate adaptively by picking the most vigorous male, and it would just so happen that he would also be the most colourful. We too found colour to correlate with a variable, plasma proteins, that may be indicative of vigour. In addition, female kestrels in our colony in mate choice experiments have consistently preferred males with high display rates (vigour?), irrespective of the degree of genetic relatedness (Duncan & Bird 1989) or experimentally induced parasite infection (Henderson *et al.* 1995). Similarly, body condition has been implicated as being important in mate choice in wild kestrels (Bortolotti & Iko 1992). The role of colour was not explored in these studies, but given the physiological benefits of carotenoids, one must, as Wallace (1895) recognized, be careful to determine whether females choose on the basis of colour or on a more direct attribute of male quality.

Whereas there is a proliferation of hypotheses concerned with the adaptive significance of colour, there has been considerably less attention devoted to the idea that colour may be non-functional. Our data could be taken to suggest more parsimonious, non-adaptive explanations for colour variation. For example, age-dependent colour patterns are common in sexually dichromatic passerines: males in their first year are not as brightly coloured as older males. Numerous adaptive explanations have been proposed to explain this (Butcher & Rohwer 1989; Savalli 1995). It is possible that for kestrels, and perhaps other species, young males may simply be physiologically limited in their ability to process carotenoids, and hence plumage or skin colouration is duller than older individuals.

Gender-biased colour may similarly be non-functional and a consequence of other physiological processes that are important to the biology of birds. The fact that shrikes, which are only black, grey, and white, showed as pronounced a gender difference in carotenoids as kestrels is compelling evidence that carotenoid metabolism can have origins and functions unrelated to colour expression. It is equally clear that many tissues (e.g. fat depots, eggs) are also pigmented by carotenoids, yet these do not evoke an adaptive explanation. It is plausible that, at least for some species, feathers and integument could be coloured independently of any action of sexual selection.

The hypothesis that sexual dichromatism was non-functional and incidental to inherent 'physiological' differences between the sexes was proposed by Alfred Russel Wallace (Cronin 1991). Wallace (1895, p. 353) recognized that whereas males of many birds are more brightly coloured than their mates, the degree of dimorphism varied greatly, with the most common case being for males 'to have the same general hue as the females, but deeper and more intensified'. Although it may be difficult to discount the role of sexual selection for extreme cases, such as house finches, the common, subtle patterns of colour variation between the sexes may be more difficult to explain except as non-functional consequences of other biochemical processes. If such processes are fundamental to avian physiology, it may explain why sexual dichromatism is so common in birds, and why reds, yellows and oranges are so pervasive.

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