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Evolution of sexual dichromatism: contribution of carotenoid- versus melanin-based coloration

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In birds, carotenoid-based plumage coloration is more dependent on physical condition and foraging abilities and less constrained developmentally than is melanin-based coloration. Thus, female mate choice for honest signals should result in more intense sexual selection on carotenoid- than on melanin-based plumage coloration. Using variation in sexual dimorphism as an indirect measure of the intensity of sexual selection, we tested the prediction that variation in sexual dimorphism is driven more by change in carotenoid-based coloration between males and females than by change in melanin-based coloration. Examination of historical changes in carotenoid- versus melanin-based pigmentation in 126 extant species of Cardueline finches supported this prediction. We found that carotenoid-derived coloration changed more frequently among congeners than melanin-based coloration. In both sexes, increase in carotenoid-based coloration score, but not in melanin-based coloration score, was strongly associated with increase in sexual dichromatism. In addition, sexual dimorphism in carotenoid-based coloration contributed more to overall dichromatism than dimorphism in melanin-based plumage. Our results supported the hypothesis that melanin-based and carotenoid-based coloration have fundamentally different signal content and suggest that combining melanin-based and carotenoid-based coloration in comparative analyses is not appropriate.

ADDITIONAL KEY WORDS:—carotenoids – Cardueline finches – melanins – sexual dichromatism – sexual selection.

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INTRODUCTION

With the resurgence of interest in sexual selection in the last twenty years, bright and contrasting plumage has once again become a central topic of discussion (Baker & Parker, 1979; Burtt, 1986; Butcher & Rohwer, 1989; Badyaev & Hill, 1999; Götmark, 1999; Senar, 1999) and has featured prominently in the discussion of various models for the evolution of sexual ornaments (Fisher, 1930; Zahavi, 1975; Hamilton & Zuk, 1982; Kirkpatrick & Ryan, 1991; Fitzpatrick, 1994; Hill, 1994a; Owens & Hartley, 1998; see Andersson, 1994 for a comprehensive review).

Given such intense interest in the evolution and maintenance of brightly coloured plumage, it is curious that so little attention has been paid to the fact that colourful or highly contrasting plumage can result from several, very different mechanisms (Gray, 1996; Owens & Hartley, 1998). First, bright coloration can be produced by either the microstructure of the feather itself, which differentially absorbs and reflects various wavelengths of light, or it can result from pigments (biochromes) deposited in the feathers during development (Fox, 1976; Brush, 1978, 1990). The three primary classes of pigments that birds use to colour feathers are carotenoids, melanins, and porphryns (Fox, 1976; Brush, 1978). In passerine birds, which will be the focus of this paper, pigment-based coloration comes exclusively from carotenoids and melanins.

Most comparative studies investigating the evolution of either sexual dichromatism or the 'brightness' of plumage, used a simple brightness or conspicuousness index that makes no distinction between structurally-based and pigment-based coloration or between melanin-based and carotenoid-based coloration (e.g. Hamilton & Zuk, 1982; Read & Harvey, 1989; Johnson, 1991; Promislow *et al.*, 1992; Martin & Badyaev, 1996). However, carotenoids and melanins are fundamentally different classes of biochromes. Melanins produce the earth tones of feathers—black, grey, brown, rufous, and yellow. They are synthesized by animals as a by-product of amino-acid catabolism (Fox, 1976). In birds, melanin deposition depends on duration of melanocyte activity in growing feather (Bowers, 1988) and the interaction of the melanocyte with the epidermis (Nickerson, 1944; Rawles, 1959). Melanin deposition appears to be under tight genetic control and is not easily affected by environmental variation, including diet (Decker & McGinnis, 1947; Fox, 1976; Buckley, 1987; but see Veiga & Puerta, 1996, Hill, 2000). Thus, plumage with melanin-based coloration may be a poor candidate for a reliable signal of phenotypic quality (Hill & Brawner, 1998). It may be that additional mechanisms (such as behavioral interactions or development stability) are required for melanin-based coloration to indicate phenotypic quality (Rohwer & Edwald, 1981; Rohwer, 1985; Møller, 1987; Fitzpatrick, 1998; reviewed in Badyaev & Hill, 1999).

In contrast to melanin pigmentation, carotenoids are responsible for the bright red, orange, and yellow coloration of plumage (Fox, 1976). No animals are known to synthesize carotenoid compounds (reviewed in Fox, 1976; Goodwin, 1984), so the capacity to produce carotenoid-based coloration is ultimately linked to ability to acquire carotenoids from food (e.g. Brush, 1978, 1990; Brush & Power, 1976; Slagsvold & Lifjeld, 1985). Moreover, higher levels of carotenoids are needed for producing conspicuous coloration than are normally consumed with food (Slagsvold & Lifjeld, 1985; Hill, 1994b, 1995a,b, 1996; Linville & Breitwish, 1997; but see Hudon, 1994; Bortolotti *et al.*, 1996). Carotenoids are also important in immune function (Lozano, 1994) and depletion of stored carotenoids because of their use in coloration may compromise the immune system (Shykoff & Widmer, 1996; Kodric-Brown, 1998; but see Hill, 1999b). Variable patterns of deposition and the diet-dependent nature of carotenoids lead to much greater environmentally-induced fluctuations in carotenoid-based coloration than in melanin-based coloration (Fox, 1976). Thus, expression of carotenoid-based plumage coloration may be a condition-dependent trait indicating the nutritional status and foraging ability of the bearer (Endler, 1983; Kodric-Brown, 1985, 1989, 1998; Milinski & Baker, 1990; Hill, 1992; Fölstad & Karter, 1992; Frischknecht, 1993; Hill *et al.*, 1994; Hill & Montgomerie, 1994; Thompson *et al.*, 1996; Nolan *et al.*, 1998).

In all avian taxa, dietary lipid soluble carotenoids are deposited in liver and in adipose tissues (Fox, 1976), thus the yellow coloration of body fat. However, species that use carotenoids to colour feathers have physiological pathways that allow and/or promote deposition of carotenoids in developing feathers (reviewed in Brush, 1990). Unlike melanins, carotenoids are deposited as ‘amorphous substances with no clear structural features’ (Brush, 1990). In addition, the specific carotenoids used in pigmentation, their concentration, and the means by which carotenoids are attached to keratin structures may vary among closely related species, among individuals in a population, and even seasonally within an individual (Fox, 1976; Brush, 1978, 1990; Hill *et al.*, 1994; Hill, 1995a,b; Stradi *et al.*, 1995, 1996).

Given that carotenoid-based plumage coloration is more dependent on condition and less constrained developmentally than melanin-based plumage coloration, adaptive models of sexual selection predict that females should use expression of carotenoid-based coloration rather than melanin-based coloration as a basis for mate choice. As a result, sexual selection is expected to be more intense on carotenoid-based coloration than on melanin-based coloration. Sexual dichromatism often has been used as a measure of the intensity of sexual selection in both interspecific (e.g. Hamilton & Zuk, 1982; Fitzpatrick, 1994; Møller & Birkhead, 1994; Owens & Hartley, 1998) and population-level studies (e.g. Price, 1984). Using variation in sexual dimorphism as an indirect measure of the intensity of sexual selection, we tested the prediction that changes in sexual dimorphism will be more closely associated with changes in the brightness of carotenoid-based coloration between males and females than by changes in melanin-based coloration. Alternatively, variation in either carotenoid- or melanin-based coloration may have evolved by natural selection acting with similar intensity on both sexes. Or, variation in carotenoid-based or melanin-based coloration may not be adaptive and may merely reflect ancestral physiological pathways of depositing pigments ingested with food or transformed during metabolism (e.g. Wallace, 1885; Bortolotti *et al.*, 1996). In these latter two cases no covariation with changes in sexual dichromatism is expected.

To test these hypotheses, we examined variation in plumage coloration in all extant Cardueline finches. Cardueline finches are especially suitable for this study for several reasons. First, biochemical studies of plumage coloration in Carduelines show that chemical origin of pigmentation (carotenoids or melanins) could be reliably inferred from plumage coloration (Stradi *et al.*, 1995, 1996 and references therein). Cardueline finches show extensive variation in sexual dimorphism and plumage coloration (Clement *et al.*, 1993; Badyaev, 1997a); contribution of carotenoid- versus melanin-based coloration to overall plumage dichromatism varies extensively in this subfamily (Appendix 1). Finally, sexual selection on carotenoid-based coloration in Cardueline finches have been subject to several intraspecific studies (Björklund, 1990; Hill, 1991, 1994b; Johnson *et al.*, 1993; Badyaev, 1994; Seutin, 1994; Drachmann, 1996).

METHODS

Scoring plumage dimorphism and coloration

Overall plumage dimorphism was estimated in two ways. First, to provide a measure of dichromatism that is independent of our data set and that is unbiased to the coloration origin (i.e. carotenoid- or melanin-based), we used the 'plumage dichromatism index' previously used for Cardueline finches (Badyaev, 1997a,b; Martin & Badyaev, 1996). To compute this index two independent observers scored dichromatism for three body regions: rump, breast, and head. Dichromatism was recorded for each body region as 0 if there was little or no dichromatism, 1 for moderate dichromatism, and 2 for high dichromatism (see Irwin, 1994). The plumage dichromatism index was then the mean sum of scores for the three regions between the two observers. There was a strong positive correlation between scorers for rump (Spearman $r=0.69$, $P<0.001$), breast ($r=0.89$, $P<0.001$), and head dichromatism ($r=0.89$, $P<0.001$), and overall brightness scores (males: $r=0.79$, $P<0.0001$, females: $r=0.65$, $P<0.001$). Similar indexes have been used in recent literature on avian coloration (e.g. Owens & Hartley, 1998). Use of such indexes allowed us to partially mitigate problems associated with subjective judgement of brightness of each sex by observers (Hunt *et al.*, 1998; Owens & Hartley, 1998; see below). The second measure of dimorphism that we used was derived from the data set used in this study and was the sum of standardized values for overall carotenoid- and melanin-based dichromatism (see below; overall plumage dichromatism hereafter). The two measures were highly correlated ($r=0.75$, $P<0.0001$).

Pigment basis of coloration in Cardueline finches was diagnosed according to Stradi *et al.* (1995, 1996). The carotenoid-based (yellow/orange/red) portion of the plumage of Cardueline finches was scored using a Colortron—a hand-held reflectance spectrophotometer (Light Source, 1994; Hill, 1998). Using the Colortron, colour measurements were taken from plates in Clement *et al.* (1993) that illustrate adult males and females (except in monomorphic species) of all extant species of Cardueline finches. Hue, saturation, and brightness scores (see below) were recorded for the crown, ventral region, and rump of illustrations of males and females of each species.

The most highly ornamented (reddest, most saturated and brightest) portion of each plumage region was scored. For instance, in most species, plumage ornamentation decreases from the throat to the undertail, but in some species a black hood obscures the throat, and in other species the undertail region may be more ornamented than the throat. The position of colour measurement on the ventral area was adjusted accordingly. For species that had no carotenoid ornamentation for a particular patch of plumage (e.g. because the head or rump was black or white) no colour measurement was made for that patch. The Colortron helps overcome problems associated with using subjective brightness scores (e.g. Hamilton & Zuk, 1982; Promislow *et al*., 1993). Difficulties arise when one tries to compare saturation and brightness of, for example, bright yellow and bright red coloration of avian plumage. Use of the continuous spectrum of the Colortron mitigates problems with deciding the degree of exaggeration of different bright colours (Hill, 1996, 1998). Many species of birds can see ultraviolet light and some plumage has peak of light reflectance extending into ultraviolet (e.g. Bennett & Cuthill, 1994). However, most carotenoid pigments, including all carotenoid pigments of Cardueline finches, reflect light primarily in the visible spectrum (e.g. Goodwin, 1973), and ignoring reflected light below 390 ηm makes little difference to these analyses. Furthermore, using the components of the Colortron-generated reflectance curve may mitigate differences between the three-cone system of the human eye and four-cones system of bird retinas (see Hill, 1998 for further discussion of the Colortron).

The use of colour plates to score plumage coloration assumes an accurate depiction of the species' coloration by the artist and then high-quality reproduction of the original plates by the publisher. To test the assumption that the colour plates in Clement *et al.* (1993) are a reasonable depiction of Cardueline finch coloration, the plumage coloration of a subset of 33 species of Cardueline finches (all species available in the Museum of Natural Sciences at Louisiana State University) was measured with the Colortron. Rump coloration was not measured for some species to prevent specimen damage. Colortron measurements of study skins and Colortron measurements taken from plates in Clement *et al.* (1993) were then compared.

The extent of black melanin pigmentation was scored independently by two zoology graduate students who were not informed of the hypothesis being tested. Each student estimated the percent of the entire male and female plumage (profile view of perched birds, wings folded) with black pigmentation from plates in Clement *et al.* (1993). There was a strong agreement between observers for the extent of melanin-based pigmentation in males (Spearman $r=0.91$, $n=132$, $P=0.0001$) and females (Spearman $r=0.88$, $n=132$, $P=0.0001$).

Data analyses

Saturation and brightness scores were given as percentiles from 0 to 100. Untransformed hue was a point along 360° colour circle that begins at red (R in the old Munsell terminology) = 0 and ends at purple-red (PR in old Munsell terminology) = 360 (see Colortron [Light Source, 1994] manual for visual depiction of the 'colour wheel'). The zero point was an arbitrary break point in the continuous colour circle. Hue values were transformed by rotating the colour circle 24° counterclockwise to account for natural break between yellow and red colours in our sample and then transformed into values proportional to arc lengths (Batschelet, 1965; Glassner, 1995). This transformation allowed calculation of the hue dimorphism as an absolute distance between male and female hue values (Batschelet, 1965). Maximum values of carotenoid-based dimorphism were then assigned to species where males have carotenoid-based coloration and females lack it. We calculated dimorphism in saturation and brightness as absolute differences between males and females for rump, breast, and crown separately. We then computed overall dimorphism in carotenoid-based plumage as the first principal component of the zero-mean transformed covariance matrix of dimorphism in hue, saturation and intensity. The eigenvector was rotated such that dimorphism in hue, saturation, and brightness all had similar loading coefficients [crown: $r=0.56$, 0.57, and 0.59 for hue, saturation and brightness correspondingly (91% of variance explained); breast: $r=0.56$, 0.57, 0.60 (84%); and rump: $r=0.61$, 0.62, 0.48 (68%)]. Overall dimorphism in carotenoid-based plumage was the sum of standardized values of dimorphism for each body part. This measure of dimorphism was highly correlated to the one obtained by simple subtraction of male and female scores for carotenoid-based plumage (see below, Spearman $r=0.75$, $P<0.0001$), but allowed for better standardization of hue, saturation, and brightness measurements.

We calculated carotenoid-based coloration of plumage as the first principal component (first eigenvector) of the zero-mean transformed covariance matrix of hue, saturation, and brightness for each sex and body part separately. Loadings were similar for each colour measure (females: crown $r=0.42$, 0.64, 0.64 for hue, saturation, and brightness correspondingly (71% of variance explained), breast $r=0.25$, 0.70, 0.70 (63%), rump $r=0.10$, 0.70, 0.70 (63%); males: crown $r=-0.10$, 0.71, 0.71 (53%), breast $r=-0.62$, 0.62, 0.52 (56%), rump $r=-0.62$, 0.57, 0.54 (61%). For each sex, overall carotenoid-based plumage coloration was computed as a sum of standardized values of the carotenoid-based coloration of each body part. Dimorphism in melanin-based coloration was calculated as normalized absolute difference between male and female percentiles.

Phylogenetic methods

Our phylogenetic hypothesis of subfamily of Cardueline finches was a consensus tree constructed by summarizing the most recent molecular, karyotypic, paleontological, osteological, and behavioral data available for each clade (Badyaev, 1997a) and is published elsewhere (Badyaev, 1997c; Tobias & Hill, 1998). We did not have consistent estimates of branch lengths because data came from studies using different methods. However, because all extant Cardueline species were included in the phylogenetic tree and plots of standardized contrasts against the variances of the untransformed contrasts showed no significant correlation, the use of equal branch lengths was justified statistically (Grafen, 1992; Garland *et al.*, 1992; Purvis & Rambaut, 1995).

To control for species relatedness within the subfamily, we analysed data by using pairwise comparisons and independent linear contrasts. In pairwise comparisons, pairs of closely related species (Appendix 2 [based on phylogenetic hypothesis presented in Badyaev, 1997a]) were ranked for overall dimorphism value and then compared with respect to a carotenoid- or melanin-based dimorphism. Any concordance with prediction was then tested with a sign test. This method provides the most direct test of concordance between overall dimorphism and dimorphism origin, because it makes fewer assumptions about phylogenetic relations among clades in the subfamily, and is statistically more powerful than ANCOVA on independent contrasts when phylogeny has unresolved nodes with multiple species (e.g. Martin & Badyaev, 1996; Badyaev, 1997b,c). We also analysed data using the independent contrast method of Felsenstein (1985) and incorporating the methods of Purvis & Garland (1993) for incompletely resolved phylogenies, based on the software described by Purvis & Rambaut (1995). By using this method we assumed that different clades are equally likely to develop similar proportional changes in each variable included in independent contrast computations. None of the regressions of absolute values of contrasts versus their estimated nodal values showed significantly different from zero slopes, thus statistically validating the assumption (Purvis & Rambaut, 1995). All regressions were forced through the origin (Garland *et al.*, 1992).

RESULTS

Reliability of plumage scores

There was a strong concordance between Colortron measurements of study skins and Colortron measurements taken from plates in Clement *et al.* (1993). Illustrations most accurately depicted true colour hue (males: Pearson $r=0.99$, $n=17$, $P<0.0001$ for crown, $r=0.99$, $n=33$, $P<0.0001$ for breast, $r=0.99$, $n=9$, $P<0.0001$ for rump; females: $r=0.47$, $n=19$, $P=0.03$ for breast). Colour saturation was also very similar between book plates and study skins (males: $r=0.51$, $P=0.04$ for crown, $r=0.57$, $P=0.0006$ for breast, no data for rump (see Methods); females: $r=0.63$, $n=16$, $P=0.009$ for breast). Colour brightness was the most different between book plates and study skins (males: $r=0.31$, $P=0.22$ for crown, $r=0.23$, $P=0.22$ for breast; females: $r=0.63$, $n=16$, $P=0.009$ for breast). Most importantly, however, the composite measures of carotenoid-based plumage that combined hue, brightness and saturation (see Methods) and that were used in our analyses, were very similar between the study skins and book plates data sets (e.g. carotenoid-based coloration of male breast: $r=0.71$, $n=33$, $P=0.04$, female breast: $r=0.41$, $n=19$, $P=0.04$). Thus, we concluded that depiction of Cardueline finch coloration on the colour plates in Clement *et al.* (1993) is suitable for our study.

Sexual dichromatism

Independent linear contrasts

Sexes were more dimorphic in carotenoid-based plumage than in melanin-based plumage (Fig. 1; Appendix 1). Dimorphism in carotenoid-based coloration accounted for $58.89 \pm 6.97\%$ (mean ± SD of $n=75$ independent contrasts of proportions listed in Appendix 1) of overall dichromatism in plumage. Dimorphism in melanin-based coloration accounted for significantly smaller proportion of overall plumage dichromatism ($41.10 \pm 6.97\%$, $t=11.98$, $P<0.0001$; Fig. 1). Sexual dimorphism in carotenoid-based coloration did not vary with sexual dimorphism in melanin-based coloration (Pearson $r=0.17$, $n=75$, $P=0.15$).

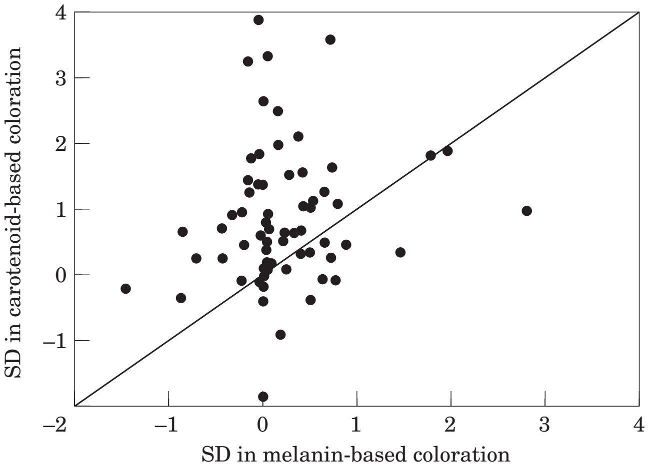

Figure 1. Relationship between sexual dimorphism (SD) in carotenoid-based coloration versus melanin-based coloration. Each point is an independent linear contrast of a proportion for each type of dimorphism in relation to overall dimorphism in plumage. Points above diagonal indicate greater contribution of dimorphism in carotenoid-based coloration than that of dimorphism in melanin-based coloration.

Males in more sexually dimorphic species had greater carotenoid scores compared to less dimorphic species (standardized regression coefficient—$b_{ST}=0.74$, $t=9.21$, $P<0.0001$ for overall plumage dichromatism measure; $b_{ST}=0.53$, $t=5.42$, $P<0.001$ for plumage dimorphism index; Fig. 2A). In males, melanin-based coloration did not vary among species with different extent of sexual dichromatism ($b_{ST}=0.02$, $t=0.195$, $P=0.85$ for overall plumage dichromatism measure; and $b_{ST}=0.17$, $t=1.89$, $P=0.08$ for plumage dimorphism index; Fig. 2B). Similarly, females' carotenoid-based coloration increased with sexual dichromatism ($b_{ST}=0.42$, $t=4.07$, $P<0.001$ for overall plumage dichromatism measure; $b_{ST}=0.45$, $t=4.42$, $P<0.001$ for plumage dimorphism index; Fig. 3A). Melanin-based coloration of females did not vary with either overall plumage dichromatism ($b_{ST}=-0.21$, $t=-2.01$, $P=0.05$ [non-significant after Bonferonni adjustment for $n=2$ comparisons] or plumage dimorphism index ($b_{ST}=-0.15$, $t=-1.49$, $P=0.15$; Fig. 3B).

Pairwise comparisons

Twenty-seven pairs of closely related species (Appendix 2) showed variation in overall plumage dichromatism; in 26 (96%) of these pairs, species with greater sexual dichromatism also had greater dimorphism in carotenoid-based coloration (sign test, $P<0.0001$). Twenty-two pairs of closely related species showed variation in both overall plumage dichromatism and dimorphism in melanin-based coloration. In 16 (73%) of the cases, the species with higher sexual dichromatism were more dimorphic in melanin-based coloration (sign test, $P=0.08$). Increase in sexual dichromatism was strongly associated with increase in carotenoid-based coloration score in both males ($\chi^2=30.89$, $P<0.0001$) and females ($\chi^2=11.34$, $P=0.0006$; Table 1). Changes

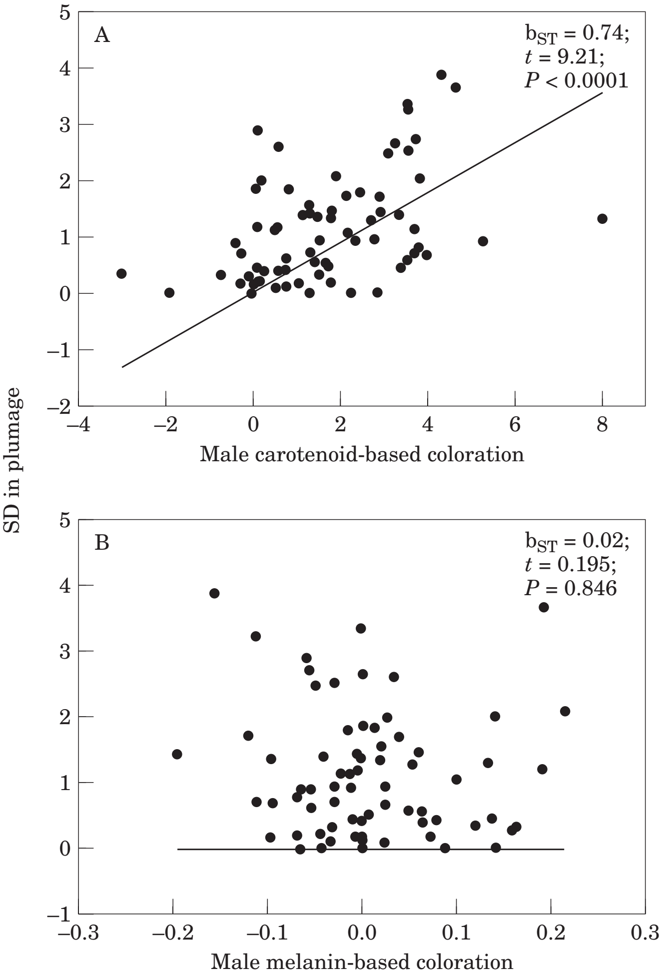

Figure 2. Regression plots of data transformed by the method of standardized independent contrasts (which controls for possible phylogenetic effects) illustrating the relationship between sexual dimorphism in plumage and (A) male carotenoid-based coloration, and (B) male melanin-based coloration.

in melanin-based coloration score did not significantly vary with sexual dichromatism in either sex (males $\chi^2 = 1.12$, females $\chi^2 = 1.22$; both $P > 0.30$; Table 1).

DISCUSSION

Most comparative studies that have investigated the conditions that promote the evolution of bright coloration or sexual dichromatism in birds have used simple, subjective brightness scales that combine carotenoid-based, melanin-based, and structural coloration (e.g. Hamilton & Zuk, 1982; Read & Harvey, 1989; Promislow *et al.*, 1992; Fitzpatrick, 1994; Martin & Badyaev, 1996). However, the fundamentally

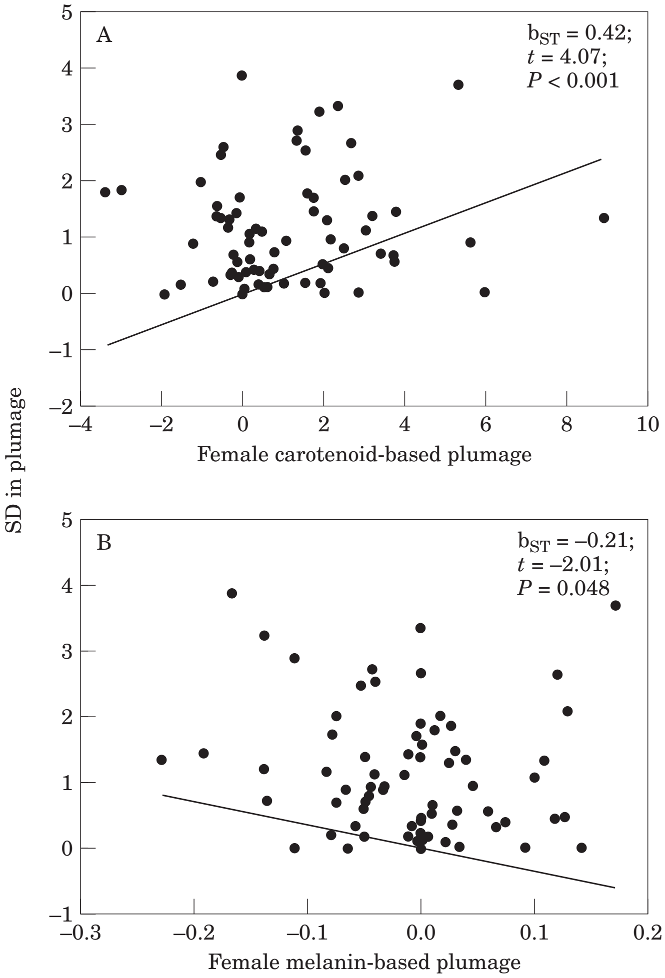

Figure 3. Regression plots of data transformed by the method of standardized independent contrasts (which controls for possible phylogenetic effects) illustrating the relationship between sexual dimorphism in plumage and (A) female carotenoid-based coloration, and (B) female melanin-based coloration.

TABLE 1. Results of pairwise comparisons of association between increase in sexual dichromatism and carotenoid versus melanin coloration in male and female Cardueline finches

Increase in sexual dichromatism	Carotenoid score			Melanin score		
	Increase	No change	Decrease	Increase	No change	Decrease
Males	22	1	3	11	6	9
Females	17	2	7	10	8	8

different natures of these types of plumage coloration lead to distinct predictions regarding their signalling properties.

Because its expression varies with access to dietary carotenoid pigments (Brush, 1978, 1990; Slagsvold & Lifjeld, 1985; Hill, 1992, 1993a; Linville & Breitwisch, 1997) and with health state (e.g. Bendich, 1989; Britton, 1995; Thompson *et al.*, 1996; Nolan *et al.*, 1998), carotenoid-based coloration is the sort of condition-dependent trait that females should use in choosing mates (Kodric-Brown & Brown, 1984; Kodric-Brown, 1985, 1989, 1998; Hill, 1991, 1996; Johnstone & Norris, 1993; Gray, 1996; reviewed in Hill, 1999a). Consistent with this prediction, in several animal species, carotenoid-based pigmentation of males is a primary criterion used by females in assessing potential mates (Kodric-Brown, 1985, 1989; Milinski & Bakker, 1990; Hill, 1991, 1994a,b; Johnson *et al.*, 1993; Bakker & Mundwiler, 1994; Sundberg, 1995). In contrast, melanin pigments are constructed from resources that are not limiting for most animals (Nickerson, 1944; Rawles, 1959; Bowers, 1988) and their expression is not generally affected by condition (Brawner, 1997; Hill & Brawner, 1998; Hill, 2000; but see Veiga, 1995). Consequently, melanin ornaments are poor predictors of condition and additional mechanisms, such as indicators of developmental stability in melanin-based patterns (e.g. Swaddle & Cuthill, 1994; Fitzpatrick, 1998) or behavioral interactions, are required for melanin-based coloration to indicate phenotypic quality (Johnstone & Norris, 1993; Gray, 1996). For example, in their study of male house sparrows (*Passer domesticus*), Veiga & Puerta (1996) found that while nutritionally-stressed males grew a smaller number of feathers with melanin-based pigmentation, the blackness of these feathers was not affected by nutritional status. Not surprisingly, few studies have found melanin-based pigmentation that appears to be ornamental (e.g. bold patches of black) to be used in female choice (e.g. Møller, 1990; Norris, 1990; Veiga, 1993). Instead, melanin pigmentation has generally been found to serve as an arbitrary badge of age or status (e.g. Rohwer, 1975, 1985; Rohwer & Edwald, 1981; Parsons & Baptista, 1980; Järvi & Bakken, 1984; Møller, 1987; Holberton *et al.*, 1989; Veiga, 1993; reviewed in Senar, 1999).

The contrast between carotenoid-based versus melanin-based coloration has been well documented in Cardueline finches. In the house finch (*Carpodacus mexicanus*) and the American goldfinch (*Carduelis tritis*), females choose males with the most saturated carotenoid-based plumage (Hill, 1991, 1994a,b; Johnson *et al.*, 1993). However, neither the size nor the darkness of a male's melanin-pigmented cap in the American goldfinch had an effect on female choice (Johnson *et al.*, 1993). Moreover, in neither American goldfinch or house finch did the redness or brightness of carotenoid-based coloration in males affect social status (Belthoff *et al.*, 1994; Johnson and Dalton, unpubl. cited in Johnson *et al.*, 1993; Belthoff & Gowaty, 1996). However, the size of melanin-based black patches of feathers had a strong effect on dominance in Eurasian siskin (*Carduelis spinus*) (Senar *et al.*, 1993). In house finches, an experimental infection with coccidians of the genus *Isospora* had a significant negative effect on carotenoid-based coloration, but no effect on melanin-based coloration (Hill & Brawner, 1998). Moreover, male house finches that survived an epidemic of mycoplasmal conjunctivitis had more saturated carotenoid-based plumage than males that died (Nolan *et al.*, 1998; see also Thompson *et al.*, 1996).

These contrasting functions of carotenoid-based and melanin-based coloration lead to different predictions regarding sexual dichromatism. Because mate choice by females exerts stronger selective pressure on males than does male choice on females (e.g. Burley, 1981; Webster, 1992; Hill, 1993b), one would expect dimorphism in the form of brighter carotenoid-based coloration in males than in females. Concurrently, because most species of Cardueline finches live in heterosexual flocks when not breeding (Newton, 1973; Clement *et al.*, 1993; Cramp & Perrins, 1994) and status signalling is important to both males and females (e.g. Nakamura, 1982), one would expect less dimorphism in melanin-based than carotenoid-based coloration.

Historical rates of change in carotenoid- versus melanin-based pigmentation in the Cardueline finch subfamily supported these predictions. Sexual dimorphism in carotenoid-based coloration was not significantly associated with sexual dimorphism in melanin-based coloration, supporting the idea that these two types of plumage coloration are under different selective pressures. These results corroborated Owens & Hartley (1998) findings that sexual dichromatisms in carotenoid and melanin coloration are correlated with different aspects of reproductive and social behaviour in birds.

We found that carotenoid-derived coloration changed more frequently among congeners than did melanin-based coloration. In both sexes, increase in carotenoid-based coloration score was strongly associated with increase in sexual dichromatism (Figs 2A and 3A, Table 1), while melanin-based coloration did not vary consistently with changes in dichromatism in either sex (Figs 2B and 3B, Table 1). It is possible that evolution of colourful carotenoid-based female plumage reflects a correlated response to intense sexual selection for colourful male plumage (e.g. Hill, 1993b). In addition, sexual dimorphism in carotenoid-based coloration contributed more to overall dichromatism than did dimorphism in melanin-based plumage (Fig. 1).

These results supported our prediction that sexual dimorphism would result from brighter carotenoid-based coloration in males than in females, while changes in melanin-based coloration between sexes would contribute less to variation in sexual dichromatism. Pairwise comparisons of association between coloration and sexual dichromatism provided particularly strong support to our predictions, because related taxa usually are similar in ecology and morphology and are likely to share similar developmental constraints on types and distribution of coloured feathers. In these comparisons of closely related congeners, changes in sexual dichromatism were strongly associated with changes in carotenoid-based coloration.

The observations from this study extend previous comparative studies on dimorphism in Cardueline finches. Badyaev (1997a) found that dimorphism of Cardueline finches decreased with increasing elevation of breeding. Harsher environmental conditions at higher elevation increase the need for parental investment by males and may decrease the intensity of sexual selection, resulting in less sexual dimorphism (Badyaev, 1993, 1997a,b). Badyaev & Ghalambor (1998) examined variation in sexual dichromatism across all extant Cardueline finches in relation to tolerance of environmental variability. Finch species that were capable of breeding over a large range of ecological conditions were also more dimorphic in plumage. The observed pattern was most concordant with interspecific differences in foraging ability and with variation in energy required for baseline metabolism (Badyaev & Ghalambor, 1998). In another comparative study of Cardueline finches, Hill (1996) found that the degree of sexual dichromatism of carotenoid-based plumage coloration increased with plumage redness, but not with amount of black pigmentation. In the study presented here, we show that sexual dichromatism results primarily from difference in carotenoid-based pigmentation, not melanin-based pigmentation. These results suggest that it is differences between the sexes primarily in carotenoid-based pigmentation rather than melanin-based pigmentation that give rise to the previously reported associations between sexual dichromatism and plumage redness, nesting elevation, and tolerance of environmental variability.

Our results confirm in Cardueline finches what Gray (1996) found in a much broader analysis of passerine birds. Using all North American passerines and then major subclades of North American passerines, he found that the amount of carotenoid pigmentation in male plumage was significantly positively associated with overall dichromatism. In contrast, but consistent with our results, he found that the amount of melanin and structural coloration in male plumage was not significantly related to overall dichromatism. Some, but not all, of the analyses on subclades supported the trends found for all North American passerines, and Gray (1996) noted that carotenoids appear to be used as ornamental signals by granivorous and insectivorous taxa (for which they are present in the diet but not overly abundant) but not used by frugivorous taxa (for which they are overly abundant in the diet) or carnivorous taxa (for which they are rare in the diet). Cardueline finches are primarily granivores and insectivores (Newton, 1973; Cramp & Perrins, 1994), so the relationship between dimorphism and carotenoid pigmentation that we found in Cardueline finches supports (at least for one additional taxon) Gray's (1996) speculation on the relationship between diet and carotenoid ornamentation. We also add to the general results of Gray (1996) that carotenoid ornamentation is significantly positively associated with degree of dichromatism in both male and female Cardueline finches, while the amount of melanin pigmentation is related to degree of dichromatism in neither sex.

Our results have two important implications. First, they support the hypothesis that melanin-based and carotenoid-based coloration have different signal content. In many species of birds, individuals have patches of feathers that are pigmented with bold melanin pigmentation as well as patches of feathers that are pigmented with bright carotenoid pigments. The need for such complex plumage patterns has not been adequately explained (Møller & Pomiankowski, 1993; Owens & Hartley, 1998). Independent signalling function of the different types of pigmentation provides an explanation for such plumage that contains both carotenoid and melanin signals (Badyaev & Hill, 1999). The second implication of our study is that combining melanin-based and carotenoid-based coloration in comparative analyses may not be appropriate (see also Gray, 1996). Melanin-based and carotenoid-based coloration should be scored separately and analysed as independent traits.

The least understood of all plumage coloration is structural coloration. There is virtually no evidence as to whether structural coloration plays a role in mate choice and/or status signalling, although Gray's (1996) results suggest it is not under as intense sexual selection as carotenoid pigmentation. Owens & Hartley (1998) found that increases in the extent of structurally based plumage dimorphism were weakly associated with increases in extra-pair paternity. These findings and our results suggest that until researchers can justify combining structural coloration and carotenoid- or melanin-based coloration, it too should be treated as an independent trait.

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APPENDIX 1

Untransformed data on contribution (%) of carotenoid- (CAR) and melanin- (MEL) based dichromatism in plumage to overall dichromatism in plumage in Cardueline finches. Transformed data were used in analyses (see Methods for details).

Species	CAR	MEL
Callacanthis burtoni	73.49	26.51
Carduelis ambigua	68.00	32.00
Carduelis atrata	0	0
Carduelis atriceps	85.71	14.29
Carduelis barbata	66.44	33.56
Carduelis cannabina	91.13	8.87
Carduelis carduelis	0	0
Carduelis chloris	85.20	14.80
Carduelis crassirostris	61.50	38.50
Carduelis cucullata	36.24	63.76
Carduelis dominicensis	57.19	42.81
Carduelis flammea	87.85	12.15
Carduelis flavirostris	80.57	19.43
Carduelis flavirostris	80.57	19.43
Carduelis hornemanni	78.90	21.10
Carduelis johannis	0	100
Carduelis lawrencei	35.34	64.66
Carduelis magellanica	77.47	22.53
Carduelis monguilloti	51.96	48.04
Carduelis notata	44.46	55.54
Carduelis olivacea	75.75	24.25
Carduelis pinus	0	0
Carduelis psaltria	43.51	56.49
Carduelis siemiradzkii	71.12	28.88
Carduelis sinica	55.23	44.77
Carduelis spinescens	78.78	21.22
Carduelis spinoides	64.68	35.32
Carduelis spinus	61.32	38.68
Carduelis tristis	72.38	27.62
Carduelis uropygialis	82.26	17.74
Carduelis xanthogastra	52.76	47.24
Carduelis yarrellii	64.06	35.94
Carduelis yemenensis	0	100
Carpodacus cassinii	100	0
Carpodacus edwardsii	100	0
Carpodacus eos	100	0
Carpodacus erythrinus	100	0
Carpodacus mexicanus	100	0
Carpodacus nipalensis	100	0
Carpodacus pulcherrimus	100	0
Carpodacus puniceus	100	0
Carpodacus purpureus	100	0
Carpodacus rhodochlamys	89.02	10.98
Carpodacus rhodochrous	95.29	4.71
Carpodacus rhodopeplus	94.96	5.04
Carpodacus roborowskii	83.94	16.06
Carpodacus roseus	100	0
Carpodacus rubescens	100	0
Carpodacus rubicilla	88.42	11.58
Carpodacus rubicilloides	100	0
Carpodacus synoicus	100	0
Carpodacus thura	100	0
Carpodacus trifasciatus	81.61	18.39
Carpodacus vinaceus	100	0
Coccothraustes abeillei	71.48	28.52
Coccothraustes coccothraustes	80.09	19.91
Coccothraustes vespertinus	90.41	9.59
Eophona migratoria	68.97	31.03
Eophona personata	0	100
Haematospiza sipahi	84.42	15.58
Leucosticte a. atrata	73.06	26.94
Leucosticte a. arctoa	0	0
Leucosticte branti	0	0
Leucosticte nemoricola	0	0
Loxia curvirostra	100	0
Loxia leucoptera	90.08	9.92
Loxia pytyopsittacus	100	0
Loxia scotica	100	0
Mycerobas affinis	58.78	41.22
Mycerobas carnipes	45.90	54.10
Mycerobas icterioides	56.07	43.93
Mycerobas melanozanthos	78.50	21.50
Pinicola enucleator	100	0
Pinicola subhimachala	100	0
Pyrrhoplectes epauletta	39.12	60.88
Pyrrhula aurantiaca	100	0
Pyrrhula erythaca	65.72	34.28
Pyrrhula erythrocephala	83.30	16.70
Pyrrhula leucogenys	100	0
Pyrrhula nipalensis	100	0
Pyrrhula pyrrhula	100	0
Rhodopechys githaginea	100	0
Rhodopechys mongolica	91.08	8.92
Rhodopechys obsoleta	30.52	69.48
Rhodopechys sanquinea	82.64	17.36
Rhynchostruthus socotranus	24.96	75.04
Serinus albogularis	0	0
Serinus ankoberensis	0	0
Serinus atrogularis	0	0
Serinus burtoni	0	0
Serinus canaria	100	0
Serinus canicollis	78.40	21.60
Serinus capistratus	29.64	70.36
Serinus citrinella	72.07	27.93
Serinus citrinelloides	66.71	33.29
Serinus citrinipectus	0	0
Serinus donaldsoni	0	0
Serinus dorsostriatus	72.87	27.13
Serinus estherae	80.88	19.12
Serinus flavigula	0	0
Serinus flaviventris	100	0
Serinus gularis	0	0
Serinus koliensis	58.99	41.01
Serinus leucopterus	0	0
Serinus leucopygius	0	0
Serinus menachensis	0	0
Serinus mennelli	0	0
Serinus mozambicus	55.27	44.73
Serinus nigriceps	24.61	75.39
Serinus pusillus	0	0
Serinus rothschildi	0	0
Serinus rufobrunneus	0	0
Serinus scotops	41.96	58.04
Serinus serinus	74.62	25.38
Serinus striolatus	0	0
Serinus sulphuratus	80.13	19.87

APPENDIX 2

Pairs of closely related species used in the pairwise comparisons

Serinus leucopterus
Serinus tristriatus

Serinus capistratus
Serinus scotops

Serinus atrogularis
Serinus leucopygius

Serinus flaviventris
Serinus donaldsoni

Serinus mozambicus
Serinus dorsostriatus

Serinus serinus
Serinus canaria

Serinus alario
Serinus nigriceps

Serinus citrinipectus
Serinus rothschildi

Serinus symonsi
Serinus albogularis

Serinus mennelli
Serinus gularis

Serinus ankoberensis
Serinus tristriatus

Serinus citrinelloides
Serinus menachensis

Serinus citrinella
Serinus canicollis

Serinus flavigula
Serinus burtoni

Serinus rufobrunneus
Serinus estherae

Carduelis flammea
Carduelis hornemanni

Carduelis flavirostris pipilans
Carduelis f. montanella

Leucosticte arctoa arctoa
Leucosticte a. atrata

Leucosticte nemoricola
Leucosticte branti

Carpodacus purpureus
Carpodacus cassinii

Carduelis cannabina
Carduelis yemenensis

Carduelis tristis
Carduelis lawrencei

Carduelis pinus
Carduelis psaltria

Carduelis johannis
Carduelis yemenensis

Carduelis chloris
Carduelis sinica

Carduelis ambigua
Carduelis spinoides

Pinicola enucleator
Pinicola subhimachala

Uragus sibiricus
Urocynchramus pylzowi

Rhodopechys githaginea
Rhodopechys mongolica

Rhodopechys sanquinea
Rhodopechys obsoleta

Eophona migratoria
Eophona personata

Mycerobas carnipes
Mycerobas icterioides

Mycerobas affinis
Mycerobas melanozanthos

Coccothraustes coccothraustes
Coccothraustes abeillei