Sexual Signals of the Male American Goldfinch

Rafael F. Rosen & Keith A. Tarvin

Department of Biology, Oberlin College, Oberlin, OH, USA

Abstract

Male American goldfinches (*Carduelis tristis*) exhibit conspicuous yellow plumage, orange bills, and black caps during the breeding season. These secondary sexual characteristics may serve as criteria by which females evaluate males as potential mates because the traits vary among individuals and are likely to be costly. We quantified plumage and bill color and cap characteristics of wild, free-ranging American goldfinches during the breeding season and tested for relationships between those features, body condition and individual genetic diversity in males. Overall, male and female goldfinches were highly sexually dichromatic, with plumage saturation and brightness and bill brightness contributing strongly to the dimorphism. Body condition decreased significantly with Julian date, even over the 2-wk period immediately prior to the onset of nesting during which we collected our color and cap measurements. Principal components describing color of the back and the bill significantly predicted date-corrected body condition based on quadratic regressions, suggesting that there is reliable information in back and bill color that females could use when choosing mates. In a subset of captive males, we found that bill hue faded from orange to yellow within 24 h of capture, suggesting that bill color may reflect short-term changes in health status or carotenoid availability. Individual genetic diversity based on a panel of eight microsatellite loci was correlated with back brightness and perhaps with cap symmetry. Based on the results of this field study, ornamentation of male American goldfinches appears to signal both long- and short-term aspects of phenotypic quality.

Introduction

Sexual signaling systems often explain conspicuous male ornaments (Andersson 1994; Johnstone 1997). In sexual signaling systems, female receivers may use information extracted from male signals as the basis for choosing mates. Females can overcome deceptive male interests by preferring signal characters that impose costs to the signaling male (Zahavi 1975; Grafen 1990) or are otherwise condition dependent (e.g. Hill 2002). Under such conditions only the highest quality males can afford to produce the signal at comparatively high levels without suffering a reduction in viability. Female preferences

for costly traits renders production of these otherwise detrimental characters by males beneficial, and these costly characters can act as honest signals whereby the strength of a male's signal indicates his quality (Johnstone 1997).

American goldfinches (*Carduelis tristis*) are socially monogamous passerine birds (Middleton 1993) that engage in extra-pair mating (Gissing et al. 1998; K.A. Tarvin, unpubl. data). During winter, male and female goldfinches are drab yellow-brown with dark wings. In spring both sexes molt into a brighter and more conspicuous yellow alternate plumage, and in males this nuptial yellow plumage is particularly bright. In addition, males also grow black feathers

on the crown to form a black 'cap.' The molt is generally completed by late Apr. or early May, although in Ohio a few individuals continue the molt into Jun. In both sexes the bill changes from a dark-grayish brown in the winter to a bright vibrant orange well before breeding begins in early Jul. The bills of males are more intensely colored than those of females during the breeding season (Middleton 1993).

Yellow goldfinch plumage derives its color from a combination of carotenoid pigments, which are deposited in growing feathers (McGraw 2004), and structural properties of the feather tissues (Shawkey & Hill 2005). Carotenoids cannot be synthesized by birds de novo, so birds must acquire carotenoids from their diet (Olson & Owens 1998). Although there are many different types of carotenoid pigments, American goldfinches of both sexes incorporate the same combination of the same specific carotenoids into their plumage. However, individuals vary in the total amount of pigment deposited (McGraw et al. 2002a), leading to males being more yellow than females. Variation also exists within the sexes, which means that some males are more yellow than others (McGraw et al. 2002a). Bill color is determined by carotenoid and melanin deposition, and has been shown to be mediated by seasonal fluctuations in testosterone (Mundinger 1972). The black areas of goldfinch plumage, including the cap, result from melanin pigments, which, unlike carotenoids, can be synthesized by birds (Prota 1992).

There are potential costs associated with the expression of each of these traits. Carotenoids have nutritional, antioxidant, and immunological benefits (Olson & Owens 1998; Blount et al. 2003; McGraw & Ardia 2003; McGraw 2005), and a bird that deposits carotenoids in its feathers or bill may incur costs because the carotenoids cannot be used elsewhere (McGraw et al. 2005). Although not as well understood, the production of structural colors appears to be costly as well (e.g. Keyser & Hill 1999; Johnsen et al. 2003; Hill et al. 2005). Research on other species indicates that melanin-based male plumage also may signal nutritional status (Jawor & Breitwisch 2003; McGraw et al. 2003; McGraw 2005; Poston et al. 2005). However, most empirical work indicates that melanin-based plumage more often acts as a reliable signal of male social status (Møller 1987; Senar et al. 1993; McGraw et al. 2002b), and may reflect the ability of an individual to deal with high levels of metal ions in the diet (McGraw 2003). Thus, a male that wears a large cap may pay social costs in the form of fighting more with other males,

and may also signal resilience to potential toxins in the diet.

Given the potential physiological costs involved in expressing yellow plumage, orange bills, and black caps, we predicted that these characters would serve as indicators of general health in male American goldfinches. We therefore tested for relationships in male goldfinches between plumage, bill color, and cap characteristics and body condition, the latter of which we assume to be generally indicative of physiological health status.

Recent studies have suggested that individual genetic diversity may be an important component of mate choice (e.g. Foerster et al. 2003; Marshall et al. 2003), and some studies have found correlations between sexual signals and measures of heterozygosity (e.g. Muller & Ward 1995; Foerster et al. 2003; Seddon et al. 2004; Reid et al. 2005). Thus, it is possible that bright plumage and bill color of goldfinches may signal individual genetic diversity, either directly or indirectly through associations between heterozygosity and other fitness parameters such as foraging ability or disease resistance. If so, females seeking genetically diverse mates may be able to use plumage or bill color to identify preferred males. We evaluated this possibility by testing for correlations between individual genetic diversity and plumage and bill coloration, cap characteristics, and body condition.

Methods

Field Methods

We captured and measured adult goldfinches from 14 Jun. through 30 Aug. 2002 at Carlisle Reservation, Lorain County, Ohio, USA (41°17′N, 82°08′W; elevation 225 m). Most individuals were caught in mist nets placed around feeders before nesting began, but a few were netted as they were attending nests later in the season. All individuals for which we analyzed color were captured and measured within a 2-wk period immediately prior to the onset of nesting (see below). We measured the mass of each captured bird to the nearest 0.1 g and wing chord to the nearest mm. We collected approx. 20 μL of blood from the brachial vein and placed it in 200 μL of lysis buffer ('Cell Lysis Solution', Gentra Systems, Minneapolis, MN, USA) for later analysis. We aged individuals as second-year (SY; that is, hatched during the previous breeding season) or after-second-year (ASY) (Pyle 1997). From 20 Jun. to 4 Jul., prior to the onset of nesting, we measured

the color of the bill, breast, and back of a subset of birds using a portable handheld reflectance spectrophotometer (see below).

We indexed body condition as the residuals from a regression of $3 \times \ln(\text{wing chord})$ vs. $\ln(\text{mass})$ (Pryke et al. 2001) based on all adult males caught during the study period. When an individual bird was captured more than once, we included only the first set of complete measurements in the regression to avoid pseudoreplication. Tarsus length is usually preferred over wing chord for calculating body condition because tarsus length is fixed in adult birds while wing chord can vary with wear and growth of flight feathers (Freeman & Jackson 1990). However, we used wing chord because we found the very short tarsi of goldfinches were difficult to measure reliably. Moreover, all the measurements used in analyses of body condition and plumage and bill color were collected within a 2-wk period. Thus, it is unlikely that significant feather wear occurred within this time frame. Consistent with this assumption, male wing chord and Julian date were neither correlated over the entire season (r = 0.039, p = 0.733, n = 80) nor over the period during which plumage color was measured (r = −0.118, p = 0.518, n = 32). Second-year birds had significantly shorter wing chords than did after-second-year birds (SY: $\bar{x}$ = 70.74 mm, SE = 0.238, n = 48; ASY: $\bar{x}$ = 71.64 mm, SE = 0.290, n = 32; $t_{66.859}$ = −2.406, p = 0.019), so we standardized wing chord across age classes by reducing values of ASY birds by the ratio of the mean values of the two age groups before calculating body condition.

Cap characteristics were measured by taking high quality digital photographs of the dorsum of the head, with the lower mandible of the bird held flat on a table. Photographs were taken in the field using a Nikon COOLPIX995 digital camera (Nikon Corporation, Tokyo, Japan), mounted at a fixed height above the table using the close focus option and maximum zoom. The total area of the cap was measured using NIH ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, MD, USA, http://rsb.info.nih.gov/ij/, 1997–2005) software's outline and area measure tools. The mean area of four replicate traces was used to minimize measurement error. The distance between the eyes was used as the unit of measurement for each picture. This had the effect of standardizing the measurements both for head size and for variation in the distance from the crown of the bird's head to the camera. We also calculated the percent difference in area of the right and left sides of the cap (hereafter, 'area

symmetry') using a slight modification of the technique of Badyaev et al. (2001). In this case, we used ImageJ to calculate the area of each side of the cap (as number of pixels), and calculated the percent difference as [(area of the right side − area of the left side)/total area], again averaging values across four replicate measurements. We estimated degree of bilateral symmetry of the overall shape of the cap (hereafter, 'shape symmetry') by having five individuals who were unfamiliar with the objectives of the study categorize the degree of cap symmetry of each bird from 1 to 3 (1 = highly symmetrical; 3 = highly asymmetrical) based on photographs of the caps with a line drawn through the culmen and extending beyond the occiput to identify the midline. We used the average of the five rankings for each bird as an index of shape symmetry.
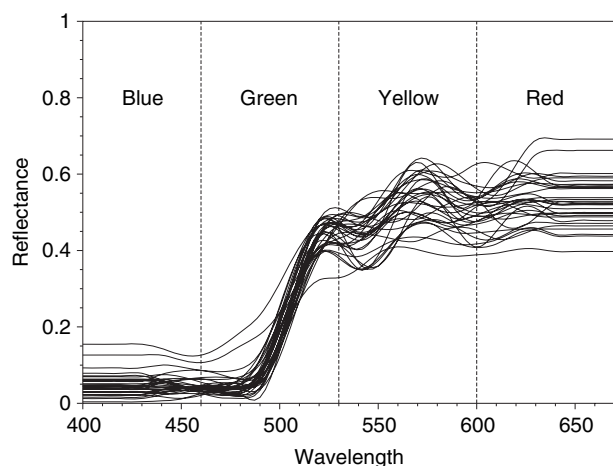
Color Assessment

Color measurements were taken in the field from 32 male and nine female American Goldfinches using a Color Savvy ColorMouse Too! Spectrophotometer (Color Savvy Systems Limited, Springboro, OH; Product no. CM2S). For each bird, four replicate readings were taken from the breast, back, and bill and an average computed for each region. Because goldfinch bills are small, we held the bill against a flat black cloth that yielded virtually no reflectance to reduce effects of extraneous light during measurements. A subset of the males for which color and morphology had been measured were held in captivity with ad libitum access to water and commercial 'thistle' (Niger) seed for 24 h (±1 h) as part of another study. We again measured bill color of these males at the end of the 24 h captive period and thereafter released them.

The ColorMouse Too! measures the amount of light an object reflects over a wavelength (λ) range of 400–700 nm in 10 nm intervals. Yellow plumage of goldfinches and other birds also generates short wavelength reflectance (<400 nm; MacDougall & Montgomerie 2003; Mays et al. 2004; Shawkey & Hill 2005), which we were unable to sample with our instrument. Although this means that we are likely to miss some potential signal information, the lack of sampling in the short wavelength range does not compromise the patterns we are able to evaluate in longer wavelength ranges (see Discussion). The reflectance scores, denoted Q_λ, range from 0 (no reflectance) to 1 (complete reflectance). For $\lambda < 420$ nm and $\lambda < 650$ nm, the reflectances began to sharply increase, sometimes rising above the theoretical limit
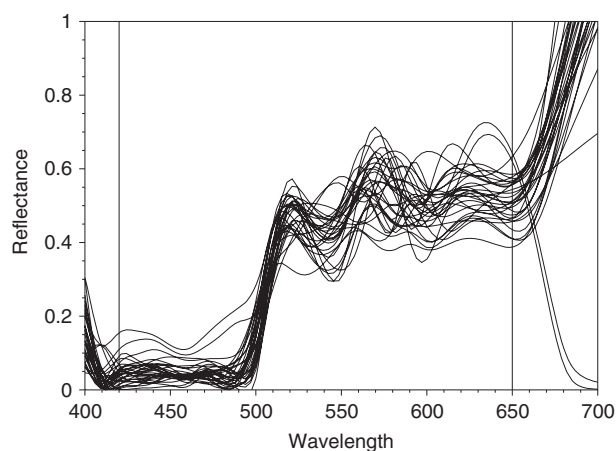
Fig. 1: An example of unprocessed reflectance data. Back measurements from 31 males are superimposed in this plot. Each curve represents the average of four replicate readings from a single individual. Note the exaggerated trajectories at the largest and smallest wavelengths. The vertical lines indicate where data were trimmed to exclude unreliable data.

of 1. It appeared that the spectrophotometer was not reliable at the extreme ends of its range, and we therefore only used data for Q_{420} through Q_{650} (Fig. 1). This trimming was unlikely to affect our analysis because we were most concerned with variation in the yellow range around 570 nm. To reduce measurement error, we recalculated a smoothed reflectance score for each λ as the average of $Q_{\lambda-10}$, Q_{λ}, and $Q_{\lambda+10}$ (Fig. 2). The first and last reflectance scores,

Q_{420} and Q_{650}, were left out of the resulting smoothed reflectance curve because there was only one adjacent reflectance score that could be used to smooth them. After trimming data outside the Q_{420} to Q_{650} range and performing the smoothing procedure, the reflectance curve for each region of each bird was composed of 22 data points from 430 to 640 nm.

We used segment classification (Endler 1990; Saks et al. 2003) to convert each reflectance curve into scores for hue, saturation (or chroma), and brightness. Segment classification breaks a reflectance curve into four segments of equal length, each of which has its own score. The wavelength ranges of the segments roughly correspond to the ranges for blue (400–460 nm), green (470–530 nm), yellow (540–600 nm), and red (610–670 nm) light. Segment scores were calculated as the amount of light reflected by the segment relative to the light reflected by the entire spectrum. The wavelength ranges of the blue and red segments each extend past the range for which we had usable data (430–640 nm). In order to create segments of equal length and still have the divisions between segments roughly correspond to those defined by Endler (1990), we used the value of Q_{430} for Q_{400}, Q_{410}, and Q_{420}. Similarly, the value of Q_{640} was used for Q_{650} through Q_{670}. We considered this an allowable inference of data because reflectance values were generally constant at wavelengths slightly higher than 430 and slightly lower than 640 (Fig. 2). These manipulations may reduce the information contained in plumage color in the blue and red range, but they do not affect the information contained in the green and yellow range. From these data, we calculated hue, saturation, and brightness following Endler (1990).

Individual Genetic Diversity

We used a panel of eight polymorphic microsatellite loci (CtA8, CtA105, CtB23, CtC16, CtC101, CtC105, CtC117, CtD108; Tarvin 2006) to assess individual genetic diversity. We extracted DNA from blood samples using a PureGene DNA Extraction Kit 5500 (Gentra Systems) following the manufacturers instructions. Polymerase chain reactions were carried out and analyzed as described in Tarvin (2006). Individual genetic diversity measures were generated from the genotypes of 128 adult American goldfinches sampled in 2002. All individuals were typed at all eight loci.

We assessed individual genetic diversity using three measures. We calculated standardized mean d^2 following Coulson et al. (1998), incorporating the

Fig. 2: An example of smoothed reflectance data from back measurements of 31 males superimposed onto a single plot. Original reflectance data were smoothed using a moving average to reduce noise. Vertical dashed lines represent boundaries used to define color segments for the transformation to brightness, saturation, and hue. See text for further details on methodology.

modification suggested by Amos et al. (2001). Standardized mean d^2 is based on the size difference between microsatellite alleles, and reflects the evolutionary divergence of alleles assuming a stepwise mutation model (Valdes et al. 1993). We used the formula presented in Amos et al. (2001) to calculate 'internal relatedness' (IR), which measures similarity of parental half-genotypes within an individual; effectively it is a measure of inbreeding (Amos et al. 2001). As a third measure, we calculated 'standardized heterozygosity' (SH) as heterozygosity at a locus (0 = homozygous, 1 = heterozygous) divided by mean heterozygosity at that locus, averaged across all loci (modified from Coltman et al. 1999). Because SH and IR were highly correlated ($r = -0.988$, $p < 0.001$, $n = 32$), we only report results for standardized mean d^2 and IR.

Statistical Analysis

We used multiple analysis of variance (MANOVA) to test for overall differences in plumage and bill characteristics between males and females and between SY and ASY males, and to identify specific variables that contributed to overall differences when they existed. In each case, Julian date was included as a covariate because plumage characteristics may change because of feather growth or wear over time. In analysis of sex, two variables (back brightness and back hue) did not meet the assumptions of MANOVA and were analyzed separately. We used principal components analysis (PCA) to extract uncorrelated factors accounting for variation in breast color, back color, bill color, and cap characteristics of males and to test for relationships among these factors and body condition. We calculated a different set of principal components for each body region separately. Preliminary analyses revealed that Julian date explained a significant amount of variation in body condition ($R^2 = 0.254$, $F_{1,30} = 9.87$, $p = 0.004$; Fig. 3) within the 2-wk period during which we collected our color and cap measurements. Therefore, we used linear and quadratic regression (see Discussion) to test for relationships between the principal components and the residuals from a regression of Julian date and body condition (hereafter referred to as date-corrected body condition). This allowed us to control for effects of date on body condition when testing for other relationships.

When testing for a change in bill color over the 24 h captive period, we extracted principal components based on bill color values from day 1 and day 2 combined into a single data set; component scores
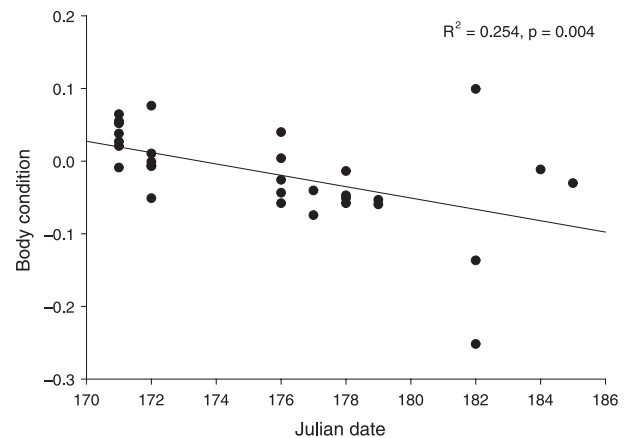
Fig. 3: Relationship between Julian date and body condition in male American goldfinches. Julian date 170 represents 19 Jun.; 186 represents 05 Jul.

from day 1 and day 2 were then compared using paired t-tests.

Sample sizes vary slightly among analyses because not all measurements were acquired for each individual bird.

Results

Sexual Dichromatism and Effects of Age on Male Characters

MANOVA indicated that males and females were significantly dichromatic (Wilk's lambda = 0.344, $F_{7,28} = 7.622$, $p < 0.001$). Male backs were brighter, more saturated, and closer to yellow than female backs, which were closer to orange (Table 1). Male breasts, on the other hand, were more saturated and brighter than those of females, but did not differ significantly in hue (Table 1). The bills of males were brighter than those of females, but the sexes did not differ in bill hue or saturation (Table 1). Males aged SY did not significantly differ overall from older males based on a MANOVA including all cap, plumage, and bill characteristics (Wilk's lambda = 0.568, $F_{12,10} = 0.633$, $p = 0.776$, $n = 12$ SY and 12 ASY males).

Relationships Among Male Characters

Within males, all color components from each region and all cap characteristics were normally distributed (1-sample Kolmogorov–Smirnov test, all $p > 0.05$). The first principal component describing breast color (Breast PC1) explained approximately 55% of the variation in breast color among males, with both saturation and hue having high factor loadings (>0.89).

Table 1: Sexual dimorphism in plumage and bill color of American goldfinches. A hue value of 0.5 corresponds to yellow, and a value of 1.5 corresponds to red. Significance values are from MANOVA including Julian date as a covariate except where noted

Character	Sex	n	$\bar{x}$	SE	p
Back brightness	M	31	0.324	0.005	<0.001[a]
	F	6	0.093	0.011	
Back saturation	M	31	0.414	0.009	<0.001
	F	6	0.286	0.015	
Back hue	M	31	0.589	0.008	0.009[a]
	F	6	0.731	0.043	
Breast brightness	M	32	0.382	0.005	0.001
	F	6	0.338	0.017	
Breast saturation	M	32	0.387	0.008	0.002
	F	6	0.302	0.021	
Breast hue	M	32	0.548	0.008	0.251
	F	6	0.532	0.017	
Bill brightness	M	31	0.108	0.004	0.013
	F	9	0.074	0.006	
Bill saturation	M	31	0.207	0.014	0.531
	F	9	0.163	0.025	
Bill hue	M	31	0.894	0.067	0.112
	F	9	0.478	0.261	

[a]Significance value from a Mann–Whitney U-test. Julian date not included as a covariate.

The second principal component (Breast PC2) explained an additional 39% of the variation, and represented brightness (factor loading = 0.98). A single principal component (Back PC1) was extracted from back color variables. It explained approx. 57% of the variation and was strongly correlated with both saturation and hue (factor loadings >0.85). The first principal component describing bill color (Bill PC1) explained approx. 52% of the variation and reflected bill brightness and hue (loadings for each >0.87). The second principal component (Bill PC2) explained an additional 34% of the variation in bill color, and reflected bill saturation (0.99). A single principal component (Cap PC1) was extracted from cap variables, primarily reflecting area symmetry (0.85) and total cap area (−0.74). Back PC1 was significantly correlated with back brightness (p = 0.047) and Breast PC1 (p < 0.001). Cap PC1 was significantly correlated with cap shape symmetry (p = 0.004). No other principal components were correlated with principal components from different body regions after a Bonferroni correction was applied (all p > 0.02; corrected α = 0.003).

Coloration and Cap Size as Indicators of Condition

Male body condition was normally distributed (1-sample Kolmogorov–Smirnov test, p > 0.05).

Linear regression did not reveal significant relationships between date-corrected body condition and any of the breast, back, bill, or cap principal components (for all coefficients, p > 0.117), or variables that were not reflected in any of the principal components (back brightness, cap shape symmetry; all p > 0.727). However, we tested for quadratic relationships between these components and date-corrected body condition under the assumption that the highest quality males are of intermediate body condition (see Discussion). Body condition was used as the dependent variable in both forms of regression to simulate whether the characters were accurate predictors of body condition. We found significant quadratic relationships between date-corrected body condition and both Back PC1 and Bill PC2, indicating that back saturation and hue and bill brightness were significant predictors of body condition (Fig. 4). Principal components describing other features of the breast, bill, and cap did not significantly predict body condition (Fig. 4), nor did the two variables that were not reflected in the principal components (for both, $R^2 < 0.029$; p > 0.727).

The difference in Bill PC2 scores of the 16 captive males from the beginning and end of the 24-h captivity period was marginally significant (paired $t_{15} = 2.124$, p = 0.051), suggesting bill hue changed during that time. Average Bill PC1 score did not differ significantly between measurement times (paired $t_{15} = 0.008$, p = 0.994), indicating that bill saturation and brightness did not change. To more easily interpret the change in bill hue, we directly compared hue values (hue on first day = 0.942, on second day = 0.455; paired $t_{15} = -2.142$, p = 0.049). This result suggests that bill hue changed from orange to more yellow during the experiment. The birds actually gained mass during the captive period (mean mass on day 1 = 12.08 g, mean mass on day 2 = 12.66 g; paired $t_{14} = 5.15$, p < 0.001), but the change in mass was not correlated with change in bill hue (r = −0.02, p = 0.943, n = 15) or with change in Bill PC2 (r = −0.24, p = 0.343, n = 15).

Neither standardized mean d^2 nor individual relatedness (IR) were correlated with any of the principal components describing potential signaling features (all p > 0.214; n = 31 for plumage and bill principal components, n = 24 for cap principal component). However, each of the variables that were not reflected in principal component factors appeared weakly related to standardized mean d^2. Back brightness (r = 0.378, p = 0.036, n = 31) increased, and cap shape symmetry (r = −0.357, p = 0.082, n = 24) marginally increased with standardized mean d^2. Neither measure of individual genetic diversity
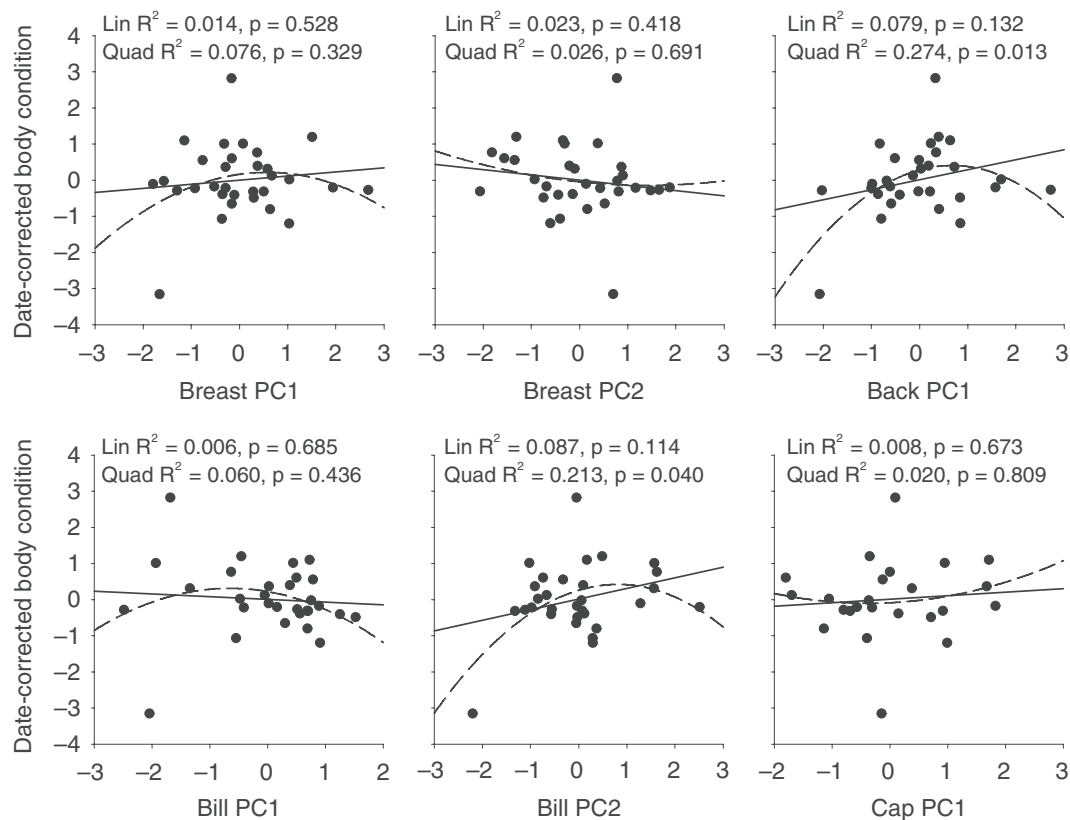
Fig. 4: Relationship between date-corrected body condition and principal components describing color of the back, breast, and bill and characteristics of the cap of male American goldfinches. Results of linear and quadratic regressions are shown in each panel. Solid lines represent the best-fit line from least-squares linear regression; dashed lines represent the fit from quadratic regression. Sample size is 32 birds for the breast regressions, 31 birds for each of the back and bill regressions, and 24 birds for the cap regression.

significantly predicted date-corrected body condition (all p > 0.12).

Discussion

Signaling Value of Plumage, Bill, and Cap

The colorful bills and plumage of male American goldfinches are conspicuous traits that are likely to have evolved by sexual selection. The relationships between these characters and body condition that we observed in our field population suggest that females may be able to assess male quality based on plumage or bill coloration. Other researchers have drawn similar conclusions based on laboratory studies. For example, McGraw & Hill (2000) demonstrated that endoparasites have a negative effect on goldfinch plumage and bill saturation, and Hõrak et al. (2004) found similar patterns in the congeneric greenfinch, *Carduelis chloris*. Moreover, McGraw et al. (2005) showed that captive goldfinches that

were nutritionally deprived during molt grew less colorful plumage. Thus, plumage and bill color may honestly reflect foraging ability or physiological ability to metabolize and assimilate costly carotenoid pigments, or similarly, they may reflect parasite status or immunocompetence, which in turn may affect body condition. Indeed, Johnson et al. (1993) found that female American goldfinches preferred brighter males in laboratory mate choice trials, and MacDougall & Montgomerie (2003) found positive assortative mating in wild goldfinches according to the intensity of yellow plumage color. Thus, although plumage of male and female goldfinches differs dramatically during the breeding season, coloration of that plumage may signal fitness traits in both sexes.

Our results are consistent with the underlying pigmentation mechanisms that control the coloration of these characters. Saturation scores have been shown to be reliable indicators of pigment abundance in both American goldfinches (McGraw & Gregory 2004) and congeneric greenfinches (Saks et al.

2003). Individual American goldfinches vary in the total amount of carotenoids they deposit in their plumage, but they do not vary in the types of carotenoids used or their relative quantities (McGraw et al. 2002a). Thus, one would expect variation in plumage saturation and for saturation to serve as an indicator of condition. We observed this pattern in our population of goldfinches, as the principal component describing variation in back color was highly correlated with saturation and significantly predicted body condition. Likewise, bill saturation also predicted body condition in our population.

Recently, Shawkey & Hill (2005) showed that yellow plumage color of goldfinches and other species is dependent to a large extent on structural properties of the feathers. Although structural properties of yellow feathers strongly affect brightness as well as short wavelength (ultraviolet) saturation, hue and saturation in the yellow to red (500–700 nm) range are primarily dependent on carotenoid pigmentation (Shawkey & Hill 2005). Therefore, as we did not detect relationships between brightness and body condition, it seems likely that the relationships we detected between hue and saturation and body condition were mediated by carotenoid pigments. Interestingly, we found that back brightness was correlated with individual genetic diversity, and thus it is possible that carotenoid pigmentation and structural components of feather color signal different aspects of individual quality. Importantly, yellow feathers also reflect short wavelength light (MacDougall & Montgomerie 2003; Mays et al. 2004; Shawkey & Hill 2005), which we were unable to detect with our spectrophotometer, and this component of color is strongly influenced by structural properties of the feathers (Shawkey & Hill 2005). Thus, it is highly possible that we missed additional important signaling information stemming from feather nanostructure. Moreover, it is possible that we missed additional features of sexual dichromatism that stem from sex-related differences in UV reflectance (Mays et al. 2004), and it is likewise possible that SY and ASY males may also exhibit such differences (e.g. Siefferman et al. 2005). Nonetheless, our lack of sampling in the UV range should not compromise our detection of longer wavelength reflectance, and therefore the relationships we found between coloration and body condition are likely only to be enhanced by more rigorous measures of reflectance in the short wavelength range.

An important element of the signal value of plumage coloration concerns the temporal nature of the information that may be signaled by plumage color.

Because carotenoid pigments are incorporated into feathers as they grow (McGraw 2004), plumage color should reflect the condition or health of the individual as it was molting, instead of its current state. Although feather and pigment degradation (or lack thereof) should allow inference of current state to some degree (McGraw & Hill 2004), it seems unlikely that plumage coloration would mirror short-term fluctuations in nutritional or disease-related condition. Bill color, however, may serve as a more refined signal of 'real-time' condition, as color change can occur relatively quickly (see also Mundinger 1972). Our observation of a change in goldfinch bill hue over a 24 h period supports this contention. However, we do not know whether the color change is because of changes in circulating pigments via the vascularized dermis (Stettenheim 1972), or to changes in melanocytes within the rhamphotheca (see Witschi 1961 for a general discussion of seasonal changes in bill color in birds).

The changes in bill hue that we observed raise further questions about the factors that determine bill color. McGraw et al. (2005) found that goldfinches subjected to food restriction during molt transported fewer carotenoids in the bloodstream and deposited fewer into the growing feathers. The finches that we maintained in captivity had ad libitum access to commercial 'thistle' (Niger) seed, and indeed they gained an average of 0.58 g (4.6% of body mass) during the 24-h captivity period. Thus, access to food per se cannot account for the change in bill color. However, it is possible that the thistle seed had a low carotenoid content, and that the birds were either unable to maintain the pre-capture levels of carotenoids in their bills during captivity, or those pigments were mobilized out of the bill for use elsewhere. Alternatively, an interesting possibility is that stress hormones (e.g. corticosterone), elevated as a result of captivity, may have interfered with the maintenance of bill color, perhaps through an interaction with testosterone. Mundinger (1972) observed changes in goldfinch bill color that were visible to the human eye (i.e. without the aid of a spectrophotometer) over a period of about a week. He found that bill color changed in concert with changes in testosterone, and inferred that the changes were because of melanin leaving the rhamphotheca. In any case, the rapid change in bill color merits further research, as the signaling content of such changes could be immense.

Cap characteristics were neither related to carotenoid-colored characters nor to body condition, a pattern also observed by McGraw & Hill (2000). These results are perhaps unsurprising given that, although

birds must acquire all their carotenoids from their diet, they can synthesize their own melanins (Prota 1992). For this reason, researchers have often assumed that melanins may not reflect health status. However, recent work has indicated that melanins may have a broader range of physiological function than previously assumed, and thus may potentially signal information about a variety of physiological conditions (McGraw 2003, 2005). Nonetheless, expression of a melanin-based cap may incur fewer nutritional or immunological costs than expression of carotenoid-based plumage and bill color (Fox 1979). Melanin-based badges are known to function as signals of male status in a variety of species, including congeneric Eurasian siskins, *Carduelis spinus* (Senar et al. 1993). Thus, there may be social costs associated with goldfinch caps that we could not detect by measuring body condition (e.g. see McGraw 2003). McGraw & Hill (2000) raised the possibility that the black cap exists simply to provide contrast for the yellow plumage in order to make the latter more noticeable. Further study into the costs associated with black goldfinch caps is needed to resolve these possibilities (McGraw 2005).

Several recent studies have found that some sexual signals may reflect individual genetic diversity (e.g. Muller & Ward 1995; Foerster et al. 2003; Seddon et al. 2004; Reid et al. 2005), which may be an important factor in populations in which inbreeding avoidance plays a role in mating strategies (for reviews, see Zeh & Zeh 1996; Tregenza & Wedell 2000; Mays & Hill 2004; Neff & Pitcher 2005). We found a correlation between one measure of individual genetic diversity (standardized mean d^2) and back brightness, as well as a marginal relationship between individual genetic diversity and cap shape symmetry. The results are compelling because heterozygosity-fitness correlations are common (e.g. see Amos et al. 2001), and individual genetic diversity may have direct or indirect effects on disease-resistance, foraging ability and other fitness-related traits. Thus, it is reasonable to expect that signals of individual genetic diversity may evolve in some systems. However, our results should be viewed with caution given the number of tests for correlations between genetic diversity indices and signal measures that we conducted, although our small sample sizes should make it difficult to detect significant relationships.

Intermediate Body Condition: Is Fatter Better?

Body condition is effectively a measure of mass that is standardized for the size of the skeletal frame on which that mass resides. We observed that quadratic regressions were better than linear regressions at predicting body condition from color characters. If color is a true index of male quality, this suggests that body condition may have a nonlinear relationship to quality. The idea that birds of intermediate condition may be of higher quality than other individuals is not unreasonable. Because mass is also a great impediment to flight, heavier birds are less effective flyers and may be at greater predation risk or incur greater metabolic costs (e.g. Witter et al. 1994; Metcalfe & Ure 1995; Adriaensen et al. 1998). Thus, it may be advantageous for a bird to keep its body mass at a level that is below its theoretical maximum, but which allows more efficient flight. Although some studies have demonstrated that dominant birds store less fat than subordinates under certain conditions (e.g. Witter & Swaddle 1995; Pravosudov et al. 1999; Krams 2002), additional research is needed to determine trends in the relationship of mass to quality (e.g. see McNamara et al. 2005). A measure of individual quality that is independent of body condition and coloration would be necessary for us to draw firmer conclusions about the signaling value of coloration in goldfinches.

Conclusions

We found evidence that ornamentation in male goldfinches reliably signals body condition at the time of molt via plumage saturation and hue, and also may signal 'real-time' fluctuations in physiological health status via short-term changes in bill hue. Moreover, we found weak but compelling evidence that plumage brightness, and perhaps features of the cap may signal information about individual genetic diversity. Together, these results suggest that male goldfinches exhibit multiple signaling ornaments that may serve as redundant signals of quality or as multiple messages about different components of quality (van Doorn & Weissing 2004).

Acknowledgements

Literature Cited

Adriaensen, F., Dhondt, A. A., Van Dongen, S., Lens, L. & Matthysen, E. 1998: Stabilizing selection on blue tit fledgling mass in the presence of sparrow hawks. Proc. R. Soc. Lond. B Biol. Sci. **265**, 1011—1016.

Amos, W., Worthington Wilmer, J., Fullard, K., Burg, T. M., Croxall, J. P., Bloch, D. & Coulson, T. 2001: The influence of parental relatedness on reproductive success. Proc. R. Soc. Lond. B Biol. Sci. **268**, 2021—2027.

Andersson, M. 1994: Sexual Selection. Princeton Univ. Press, Princeton, NJ, USA.

Badyaev, A. V., Hill, G. E., Dunn, P. O. & Glen, J. C. 2001: Plumage color as a composite trait: developmental and functional integration of sexual ornamentation. Am. Nat. **158**, 221—235.

Blount, J. D., Metcalfe, N. D., Birkhead, T. R. & Surai, P. F. 2003: Carotenoid modulation of immune function and sexual attractiveness in zebra finches. Science **300**, 125—127.

Coltman, D. W., Pilkington, J. G., Smith, J. A. & Pemberton, J. M. 1999: Parasite-mediated selection against inbred Soay sheep in a free-living island population. Evolution **53**, 1259—1267.

Coulson, T. N., Pemberton, J. M., Albon, S. D., Beaumont, M., Marshall, T. C., Slate, J., Guinness, F. E. & Clutton-Brock, T. H. 1998: Microsatellites reveal heterosis in red deer. Proc. R. Soc. Lond. B Biol. Sci. **265**, 489—495.

van Doorn, G. S. & Weissing, F. J. 2004: The evolution of female preferences for multiple indicators of quality. Am. Nat. **164**, 173—186.

Endler, J. A. 1990: On the measurement and classification of colour in studies of animal colour patterns. Biol. J. Linn. Soc. Lond. **41**, 315—352.

Foerster, K., Delhey, K., Johnsen, A., Lifjeld, J. T. & Kempenaers, B. 2003: Females increase offspring heterozygosity and fitness through extra-pair matings. Nature **425**, 714—717.

Fox, D. L. 1979: Biochromy: Natural Coloration of Living Things. Univ. of California Press, Berkeley and Los Angeles.

Freeman, S. & Jackson, W. M. 1990: Univariate metrics are not adequate to measure body size. Auk **107**, 69—74.

Gissing, G. J., Crease, T. J. & Middleton, A. L. A. 1998: Extrapair paternity associated with renesting in the American Goldfinch. Auk **115**, 230—234.

Grafen, A. 1990: Biological signals as handicaps. J. Theor. Biol. **44**, 517—546.

Hill, G. E. 2002: A Red Bird in a Brown Bag: The Function and Evolution of Ornamental Plumage Coloration in the House Finch. Oxford Univ. Press, Oxford.

Hill, G. E., Doucet, S. M. & Bucholz, R. 2005: The effect of coccidial infection on iridescent plumage coloration in wild turkeys. Anim. Behav. **69**, 387—394.

Hõrak, P., Saks, L., Karu, U., Ots, I., Surai, P. F. & McGraw, K. J. 2004: How coccidian parasites affect health and appearance of greenfinches. J. Anim. Ecol. **73**, 935—947.

Jawor, J. M. & Breitwisch, R. 2003: Melanin ornaments, honesty, and sexual selection. Auk **120**, 249—265.

Johnsen, A., Delhey, K., Andersson, S. & Kempenaers, B. 2003: Plumage colour in nestling blue tits: sexual dichromatism, condition dependence and genetic effects. Proc. R. Soc. Lond. B Biol. Sci. **270**, 1263—1270.

Johnson, K., Dalton, R. & Burley, N. 1993: Preferences of female American goldfinches (*Carduelis tristis*) for natural and artificial male traits. Behav. Ecol. **4**, 138—143.

Johnstone, R. A. 1997: The Evolution of Animal Signals. In: Behavioural Ecology, 4th edn (Krebs, J. R. & Davies, N. B., eds). Blackwell Science, Oxford, pp. 155—178.

Keyser, A. J. & Hill, G. E. 1999: Condition-dependent variation in the blue-ultraviolet coloration of a structurally based plumage ornament. Proc. R. Soc. Lond. B Biol. Sci. **266**, 771—777.

Krams, I. 2002: Mass-dependent take-off ability in wintering great tits (*Parus major*): comparison of top-ranked adult males and subordinate juvenile females. Behav. Ecol. Sociobiol. **51**, 345—349.

Møller, A. P. 1987: Variation in male house sparrows *Passer domesticus*: evidence for status signaling. Anim. Behav. **35**, 1637—1644.

MacDougall, A. K. & Montgomerie, R. 2003: Assortative mating by carotenoid-based plumage colour: a quality indicator in American goldfinches, *Carduelis tristis*. Naturwissenschaften **90**, 464—467.

Marshall, R. C., Buchanan, K. L. & Catchpole, C. K. 2003: Sexual selection and individual genetic diversity in a songbird. Proc. R. Soc. Lond. B Biol. Sci. Suppl. **2**, S248—S250.

Mays, H. L. & Hill, G. E. 2004: Choosing mates: good genes versus genes that are a good fit. Trends Ecol. Evol. **19**, 554—559.

Mays, H. L., Jr, McGraw, K. J., Ritchison, G., Cooper, S., Rush, V. & Parker, R. S. 2004: Sexual dichromatism in the yellow-breasted chat *Icteria virens*: spectrophotometric analysis and biochemical basis. J. Avian Biol. **35**, 125—134.

McGraw, K. J. 2003: Melanins, metals, and mate quality. Oikos **102**, 402—406.

McGraw, K. J. 2004: Colorful songbirds metabolize carotenoids at the integument. J. Avian Biol. **35**, 471—476.

McGraw, K. J. 2005: The antioxidant function of many animal pigments: are there consistent health benefits of sexually selected colourants? Anim. Behav. **69**, 757—764.

McGraw, K. J. & Ardia, D. R. 2003: Carotenoids, immunocompetence, and the information content of sexual colors: an experimental test. Am. Nat. **162**, 704—712.

McGraw, K. J. & Gregory, A. J. 2004: Carotenoid pigments in male American goldfinches: what is the optimal biochemical strategy for becoming colourful? Biol. J. Linn. Soc. Lond. **83**, 273—280.

McGraw, K. J. & Hill, G. E. 2000: Differential effects of endoparasitism on the expression of carotenoid- and melanin-based ornamental coloration. Proc. R. Soc. Lond. B Biol. Sci. **267**, 1525—1531.

McGraw, K. J. & Hill, G. E. 2004: Plumage color as a dynamic trait: carotenoid pigmentation of male house finches (*Carpodacus mexicanus*) fades during the breeding season. Can. J. Zool. **82**, 734—738.

McGraw, K. J., Hill, G. E., Stradi, R. & Parker, R. S. 2002a: The effect of dietary carotenoid access on sexual dichromatism and plumage pigment composition in the American goldfinch. Comp. Biochem. Physiol. B Biochem. Mol. Biol. **131**, 261—269.

McGraw, K. J., Mackillop, E. A., Dale, J. & Hauber, M. E. 2002b: Different colors reveal different information: how nutritional stress affects the expression of melanin- and structurally based ornamental plumage. J. Exp. Biol. **205**, 3747—3755.

McGraw, K. J., Dale, J. & Mackillop, E. A. 2003: Social environment during molt and the expression of melanin-based plumage pigmentation in male house sparrows *Passer domesticus*. Behav. Ecol. Sociobiol. **53**, 116—122.

McGraw, K. J., Hill, G. E. & Parker, R. S. 2005: The physiological costs of being colourful: nutritional control of carotenoid utilization in the American goldfinch, *Carduelis tristis*. Anim. Behav. **69**, 653—660.

McNamara, J. M., Barta, Z., Houston, A. I. & Race, P. 2005: A theoretical investigation of the effect of predators on foraging behaviour and energy reserves. Proc. R. Soc. Lond. B Biol. Sci. **272**, 929—934.

Metcalfe, N. B. & Ure, S. E. 1995: Diurnal variation in flight performance and hence potential predation risk in small birds. Proc. R. Soc. Lond. B Biol. Sci. **261**, 395—400.

Middleton, A. L. A. 1993: American Goldfinch. No. 80. In: The Birds of North America (Poole, A. & Gill, F., eds). American Ornithologists' Union, Philadelphia, pp. 1—24.

Muller, G. & Ward, P. I. 1995: Parasitism and heterozygosity influence the secondary sexual characters of the European minnow, *Phoxinus phoxinus* (L) (Cyprinidae). Ethology **100**, 309—319.

Mundinger, P. C. 1972: Annual testicular cycle and bill color change in the eastern American goldfinch. Auk **89**, 403—419.

Neff, B. D. & Pitcher, T. E. 2005: Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. Mol. Ecol. **14**, 19—38.

Olson, V. A. & Owens, I. P. F. 1998: Costly sexual signals: are carotenoids rare, risky or required? Trends Ecol. Evol. **13**, 510—514.

Poston, J. P., Hasselquist, D., Stewart, I. R. K. & Westneat, D. F. 2005: Dietary amino acids influence plumage traits and immune responses of male house sparrows, *Passer domesticus*, but not as expected. Anim. Behav. **70**, 1171—1181.

Pravosudov, V. V., Grubb, T. C., Doherty, P. F., Bronson, C. L., Pravosudova, E. V. & Dolby, A. S. 1999: Social dominance and energy reserves in wintering woodland birds. Condor **101**, 880—884.

Prota, G. 1992: Melanins and Melanogenesis. Academic Press, San Diego, California.

Pryke, S. R., Andersson, S. & Lawes, M. J. 2001: Sexual selection of multiple handicaps in the red-collared widowbird: female choice of tail length but not carotenoid display. Evolution **55**, 1462—1563.

Pyle, P. 1997: Identification Guide to North American Birds, Part 1. Slate Creek Press, Bolinas, CA.

Reid, J. M., Arcese, P., Cassidy, A., Marr, A. B., Smith, J. N. M. & Keller, L. F. 2005: Hamilton and Zuk meet heterozygosity? Song repertoire size indicates inbreeding and immunity in song sparrows (*Melospiza melodia*). Proc. R. Soc. Lond. B Biol. Sci. **272**, 481—487.

Saks, L., McGraw, K. J. & Hõrak, P. 2003: How feather colour reflects its carotenoid content. Funct. Ecol. **17**, 555—561.

Seddon, N., Amos, W., Mulder, R. A. & Tobias, J. A. 2004: Male heterozygosity predicts territory size, song structure and reproductive success in a cooperatively breeding bird. Proc. R. Soc. Lond. B Biol. Sci. **271**, 1823—1829.

Senar, J. C., Camerino, M., Copete, J. L. & Metcalfe, N. B. 1993: Variation in the black bib of the Eurasian siskin (*Carduelis spinus*) and its role as a reliable badge of dominance. Auk **110**, 924—927.

Shawkey, M. D. & Hill, G. E. 2005: Carotenoids need structural colours to shine. Biol. Lett. **1**, 121—124.

Siefferman, L., Hill, G. E. & Dobson, F. S. 2005: Ornamental plumage coloration and condition are dependent on age in eastern bluebirds *Sialia sialis*. J. Avian Biol. **36**, 428—435.

Stettenheim, P. 1972: The integument of birds. In: Avian Biology, Volume II (Farner, D. S., King, J. R. & Parkes, K. C., eds). Academic Press, New York, pp. 2—63.

Tarvin, K. A. 2006: Polymorphic microsatellite loci from the American goldfinch (*Carduelis tristis*) and their cross-amplification in a variety of passerine species. Mol. Ecol. Notes. **6**, 470—472.

Tregenza, T. & Wedell, N. 2000: Genetic compatibility, mate choice and patterns of parentage: invited review. Mol. Ecol. **9**, 1013—1027.

Valdes, A. M., Slatkin, M. & Feimer, N. B. 1993: Allele frequencies at microsatellite loci: the stepwise mutation model revisited. Genetics. **133**, 737—749.

Witschi, E. 1961: Sex and secondary sexual characters. In: Biology and Comparative Physiology of Birds, Volume II (Marshall, A. J., ed.). Academic Press, New York, pp. 115—168.

Witter, M. S. & Swaddle, J. P. 1995: Dominance, competition, and energetic reserves in the European starling, *Sturnus vulgaris*. Behav. Ecol. **6**, 343—348.

Witter, M. S., Cuthill, I. C. & Bosner, R. H. C. 1994: Experimental investigations of mass dependent predation risk in the European starling, *Sturnus vulgaris*. Anim. Behav. **48**, 201—222.

Zahavi, A. 1975: Mate selection - a selection for a handicap. J. Theor. Biol. **3**, 205—214.

Zeh, J. A. & Zeh, D. W. 1996: The evolution of polyandry I: intragenomic conflict and genetic incompatibility. Proc. R. Soc. Lond. B Biol. Sci. **263**, 1711—1717.