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Differential effects of endoparasitism on the expression of carotenoid- and melanin-based ornamental coloration

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The striking diversity of sexual dimorphisms in nature begs the question: Why are there so many signal types? One possibility is that ornamental traits convey different sets of information about the quality of the sender to the receiver. The colourful, pigmented feathers of male birds seem to meet the predictions of this hypothesis. Evidence suggests that carotenoid pigmentation reflects the nutritional condition of males during moult, whereas in many instances melanin pigmentation is a reliable indicator of social status. However, as of yet there have been no experimental tests to determine how these two ornament types respond to the same form of environmental stress. In this study, we tested the effect of endoparasitic infection by intestinal coccidians (*Isospora* sp.) on the expression of both carotenoid- and melanin-based ornamental coloration in captive male American goldfinches (*Carduelis tristis*). We found that the carotenoid-based plumage and bill coloration of parasitized males was less saturated than that developed by unparasitized males, but that the brightness and size of melanin-based black caps did not differ between the groups. These findings provide the most robust empirical support to date for the notion that carotenoid and melanin ornaments reveal different information to conspecifics.

Keywords: American goldfinch; *Carduelis tristis*; coccidia; honest advertisement; *Isospora*; plumage coloration

1. INTRODUCTION

Since Darwin (1871) developed his theory of sexual selection to explain the remarkable sex differences in size, shape, colour and behaviour that exist among animals in nature, biologists have been fascinated by the evolution of sexually dimorphic traits. Yet the information content of ornate male characteristics has been subject to critical empirical testing only in the last few years. Visual signals, and in particular the colourful feathers that are displayed by male birds, have been the focus of many tests of the signalling function of ornamental traits (e.g. Butcher & Rohwer 1989; Savalli 1995).

In many of the first attempts to understand the origin and maintenance of brightly coloured plumage, coloration was characterized as a single trait (e.g. Hamilton & Zuk 1982; Johnson 1991). However, the bright colours in feathers can be produced by several different mechanisms that may follow completely different evolutionary trajectories (Fox & Vevers 1960). The most well studied of these forms of plumage coloration is pigment-based ornamentation, and plumage pigmentation can result from the deposition of either melanin pigments, which are black or brown in coloration, or carotenoid pigments, which vary in hue from red to orange to yellow (Fox 1976; Brush 1978).

Most of the early studies that evaluated the information content of variation in avian plumage pigmentation focused on melanin-based coloration in males. Rohwer (1975) proposed the idea that variation in colour or patch size may function as a badge of social status (the 'status signalling' hypothesis), and it has been demonstrated subsequently in a number of species that melanin-based ornaments function as indicators of social rank (reviewed in Senar 1999). Interestingly, carotenoid-based plumage coloration has been largely ignored in tests of status signalling. Results from a few correlational studies suggest that carotenoid coloration may also be related to dominance (Marler 1955; Searcy 1979; Shawcross & Slater 1984; Eckert & Weatherhead 1987; Maynard Smith & Harper 1988; McGraw & Hill 2000*a*), but the only two experimental studies on carotenoid-based plumage brightness and aggression suggest that these ornaments are unreliable predictors of social status (Wolfenbarger 1999; McGraw & Hill 2000*b*).

Unlike those studies that have considered the behavioural mechanisms underlying bright plumage, which have been dominated by species with melanin pigmentation, studies on how ornamental plumage is produced have focused on species that deposit carotenoid pigments. Carotenoids cannot be synthesized *de novo* by vertebrates, so they must be ingested in their diet before being absorbed, transported, processed and deposited in feathers (Brush 1981; Goodwin 1984). Such acquisitional and usage demands make carotenoid ornaments particularly sensitive to environmental factors (Hill 1995, 1996, 1999; Olson & Owens 1998). Several studies have documented the influence of ecological stressors (e.g. food or pigment limitation, parasitic infection) on the expression of carotenoid-based coloration (Slagsvold & Lifjeld 1985; Hill 1992, 1993, 2000; Hill & Montgomerie 1994; Olson 1996; Linville & Breitwisch 1997; Brawner *et al*. 2000), corroborating the assertion that carotenoid ornaments

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reflect the health and condition of males. In contrast, although a handful of recent studies suggest that nutritional stress during moult may affect the development of melanin-based coloration (Slagsvold & Lifjeld 1992; Veiga & Puerta 1996; Otter & Ratcliffe 1999), no experimental studies have been conducted to consider the proximate bases of variation in melanin-based ornamental plumage pigmentation.

The pattern that emerges from previous studies of carotenoid- and melanin-based plumage coloration is clear. Melanin and carotenoid pigmentation seem to communicate different information to conspecifics, with carotenoid pigmentation representing the physiological condition of males during moult and melanin coloration indicating social status. However, these conclusions can be drawn only from comparisons of information content across species. As of yet, no study has considered the signalling functions of carotenoids and melanins in a species in which both types of ornaments are present. As a result, the goal of our study was to examine how both carotenoid and melanin ornamentation may act as indicators of health and condition in the American goldfinch (*Carduelis tristis*). Males of this passerine species display carotenoid-based plumage (yellow) and bill (orange) coloration as well as a melanin-based black cap during the breeding season, whereas breeding females are drab yellow and olive in coloration and do not possess a black cap (Middleton 1993). Females prefer to mate with males displaying the brightest carotenoid-based coloration (Johnson *et al*. 1993), but nothing is known of the signalling function of melanin ornamentation in goldfinches.

One of the assumptions of the Hamilton & Zuk (1982) hypothesis for parasite-mediated sexual selection is that the expression of plumage signals that indicate the health of males should be depressed by parasitic infection. Thus, we specifically tested the effect of endoparasitism on the expression of ornamental coloration in male goldfinches. We felt that intestinal coccidians from the genus *Isospora* would be appropriate parasites for testing this assumption because coccidia commonly infect a number of songbird species (zebra finch (*Taeniopygia guttata*, Helman *et al*. 1984), Nashville warbler (*Vermivora ruficapilla*, Swayne *et al*. 1991), northern cardinal (*Cardinalis cardinalis*, Baker *et al*. 1996) and black siskin (*Carduelis atrata*, Giacomo *et al*. 1997)) and because there are clear physiological mechanisms by which they compromise the condition of moulting males. These endoparasites directly inhibit the uptake of essential dietary components, including carotenoids, in the gastrointestinal tracts of chickens (Ruff *et al*. 1974; Augustine & Ruff 1983; Allen 1987, 1988, 1992) and consequently depress bright carotenoid-based pigmentation in poultry ('pale bird syndrome'; Tyczkowski *et al*. 1991) and male house finches (*Carpodacus mexicanus*, Brawner *et al*. 2000).

Olson (1996) found that more than 70% of wild male American goldfinches are infected with *Isospora* sp. during moult and that coccidiosis depresses bright carotenoid-based plumage and bill coloration in this species. However, she did not consider the effect of this parasite on the melanin-based ornamental coloration of male goldfinches. Thus, before males began their pre-alternate moult, we allowed experimental groups of captive male goldfinches to become infected with isosporan coccidians; we treated control groups with a coccidiostatic drug to keep individuals free of infection. After all males had completed moult, we quantified the expression of melanin- and carotenoid-based pigmentation.

2. METHODS

Between 15 and 24 January 1999, we captured 30 male goldfinches in basket traps at thistle feeders from each of two sites in Lee County, AL, USA, that were separated by 10 km. At capture, males were aged as either 'first year' or 'after first year' based on plumage characteristics (Middleton 1974). We randomly divided the birds captured from each site into two groups and placed the four groups of 15 males into separate outdoor cages (3.7 m long × 1.5 m wide × 2.4 m high). Birds were fed *ad libitum* diets of thistle, sunflower kernels and water for the duration of the study, and food and water were changed every second day. Previous work with captive male goldfinches (G. E. Hill, unpublished data) indicated that, even when diets were not supplemented with specific carotenoids, males would still moult into a drab yellow plumage coloration due to the small quantities of carotenoids present in the seeds they were fed (K. J. McGraw and R. Stradi, unpublished data). Two dishes of food and water were placed in each cage to minimize resource defence by aggressive males and thus to allow all males equal and unlimited access to both food and water. Water was treated with 6.6 drops per litre of Premium Multi-Drops^{TM} high-potency multivitamins (Eight in One, Pet Products Inc., Hauppauge, NY, USA) and 0.26 g l^{-1} of sulphadimethoxine (a sodium salt used to suppress coccidiosis; Brawner *et al*. 2000). To standardize and suppress coccidial infections that males may have brought into captivity with them, all males received sulphadimethoxine-treated water for at least one week prior to the start of the experiment. No males were infected with obvious ectoparasites (avian pox, ticks or mycoplasmal conjunctivitis) at any time during our study.

From our four groups of males, we established two infected groups and two uninfected groups and performed replicate experiments (hereafter referred to as replicates 1 and 2) testing the same hypothesis. Thus, each replicate was composed of 15 treatment males and 15 control males that were captured from the same site. The two uninfected groups of males received sulphadimethoxine-treated water throughout the course of the experiment (dosage identical to that given above), while the two infected groups remained untreated. Previous work with captive goldfinches demonstrated that these birds would become infected with coccidians from the genus *Isospora* even in the absence of specific inoculations (G. E. Hill, personal observation). Experimental treatments began on 1 February 1999, birds began moulting in mid-March and treatments continued through to the end of moult (1 June 1999).

To be sure that our treatment groups differed with respect to endoparasite load, we assessed the degree of infection for all males during moult by obtaining faecal samples on 16 April 1999 and estimating the number of coccidial oocysts shed in the faeces. Faeces were sampled in the afternoon, when oocyst shedding is greatest (Brawner & Hill 1999). Samples were stored in potassium dichromate to preserve them until laboratory analyses could be completed. Faecal float and microscope-slide preparations follow those used by Brawner & Hill (1999). Parasite load was estimated on an integer scale from 0–5 using logarithmic designations: 0, no oocysts present; 1, between one and ten oocysts present; 2, 11–100 oocysts; 3, 101–1000 oocysts; 4, 1001–10 000 oocysts; and 5, > 10 000 oocysts. These data were

Table 1. *Means ± 1 s.d. (sample sizes in parentheses) of parasite loads, food consumption rates, wing-chord lengths, body masses and ages for all four groups of captive male goldfinches*

	replicate 1		replicate 2	
	infected	uninfected[a]	infected	uninfected[a]
level of parasitic infection[b]	4.60 ± 0.70 (15)	0.14 ± 0.36	3.70 ± 1.30 (15)	0.15 ± 0.37
feeding rate (g bird^{-1} day^{-1})[c]	1.62 ± 0.40	2.07 ± 0.35	1.63 ± 0.21	2.04 ± 0.32
wing-chord length (mm)	71.17 ± 1.73 (9)	70.20 ± 1.66	70.96 ± 1.79 (12)	69.8 ± 1.90
body mass (g)	11.93 ± 1.67 (4)	14.82 ± 1.57	13.98 ± 1.12 (4)	14.86 ± 0.99
age[d]	0.22 (9)	0.75	0.50 (12)	0.60

[a] $n = 15$ for all measures (except feeding rate).
[b] Estimated on an integer scale from 0–5 as the number of coccidial oocysts shed in the faeces.
[c] Eight daily feeding rates were used to calculate mean feeding rate for each cage, but when feeding rates were determined the number of males in each cage ranged from 7–15.
[d] Proportion of first-year birds in the group.

not normally distributed (Shapiro–Wilk *W*-test, $p < 0.05$), so we compared parasite loads of infected and uninfected birds using Mann–Whitney *U*-tests.

Parasitic infection may also affect the feeding behaviour of males, and we therefore quantified food-consumption rates of birds in each cage during four separate bouts. These bouts were dispersed opportunistically throughout the course of moult. At 15.00, we removed all old food and placed 100 g of fresh sunflower kernels in each cage. Food was placed in a single 0.75 m^2 container, which was large enough to allow many males access to the food at any one time. No other food was available to the birds during these trials; concrete floors were swept clean and all vegetation was at least 1 m away from the cages. Pilot work showed that individual male goldfinches consume *ca.* 2 g of food in a 24-h period (sunset–sunset; K. J. McGraw, personal observation), so with 15 birds per cage and 100 g of food initially available over a 48-h period it is safe to assume that food was never limiting for any individual. To determine food-consumption rates in each cage, we measured how much food (to the nearest 0.1 g) remained in the container after 24 h and then again after 48 h. For each 24-h interval, we divided total grams consumed by all males in the cage by the number of males in the cage to obtain the number of grams consumed per bird per day. Four bouts, with two days per bout, yielded a total of eight days for which we obtained feeding rates for each cage. Feeding rates within each replicate were normally distributed (Shapiro–Wilk *W*-tests, all $p > 0.3$) and had equal variances (equality-of-variances *F*-tests, all $p > 0.3$), so we compared mean feeding rates of treatment groups using paired *t*-tests.

After the birds had completed moult, we measured flattened wing chord length to the nearest millimetre and body mass to the nearest 0.1 g. We scored carotenoid- and melanin-based pigmentation using a Colortron^{TM} reflectance spectrophotometer (Light Source, San Rafael, CA, USA). With each reading, the Colortron^{TM} provides tristimulus scores: 1, hue; 2, saturation (chroma, intensity); and 3, brightness (blackness, tone) (Hill 1998). We quantified carotenoid ornamentation as per cent saturation because this unit of measurement captures significant colour variation in species in which males vary little in hue (Ryan *et al*. 1994; Figuerola & Gutiérrez 1998). Carotenoid-based plumage coloration of male goldfinches was computed as the average of six saturation scores (three dorsal measurements and three ventral measurements) and we took individual readings from the upper and lower mandible to assess mean bill saturation. As black melanin pigmentation varies little in colour or purity, cap coloration was measured as per cent brightness. Cap brightness was determined by averaging Colortron^{TM} scores from the left and right sides of the head. Using calipers we also measured the diameter (front to back) of the black cap to the nearest 0.1 mm.

We were able to score ornamental coloration for only eight living males in the two infected groups because 22 males in the infected groups died during the experiment (11 in each of the two parasitized groups). Some of the males that died had nearly completed growth of their carotenoid and/or melanin ornaments, however, so we quantified carotenoid saturation for five additional males in the infected group of replicate 1 and seven in replicate 2. We also took post-mortem measurements of cap size and brightness for three males in the infected group of replicate 1 and six in replicate 2. We chose not to score the bill coloration of deceased animals because it is androgen dependent (Mundinger 1972) and fades soon after death. We found no differences between living and dead infected birds for any of our plumage colour measures (Mann–Whitney *U*-tests, all $p > 0.5$), and so we included both sets of infected males in our analyses. All plumage data were normally distributed (Shapiro–Wilk *W*-tests, all $p > 0.1$) and had equal variances (equality-of-variances *F*-tests, all $p > 0.1$), so we used unpaired *t*-tests to compare the ornaments of treatment groups within replicates. Because we tested the same biological hypothesis with multiple statistical tests, we used sequential Bonferroni corrections (Rice 1989) to account for the two independent comparisons we made in each replicate (minimum $\alpha = 0.025$).

Although males were assigned randomly to treatment groups, we tested whether groups differed in age or body size because such differences might confound treatment effects on ornamental colour display. In both replicates there were no significant differences in wing-chord length between infected and uninfected males (replicate 1, Mann–Whitney $U = 88$, $p = 0.21$; replicate 2, $U = 121$, $p = 0.13$; table 1). However, the proportion of first-year birds differed between infected and uninfected males in one of our replicates (replicate 1, $\chi^2 = 31.5$, $p < 0.01$; replicate 2, $\chi^2 = 0.63$, $p = 0.43$; table 1). Nevertheless, this difference between treatment groups should not have influenced plumage coloration because Olson (1996) found no age-related plumage trends among either wild or captive male goldfinches in Ontario, Canada. Similarly, when we separately considered infected birds and uninfected birds, age was unrelated to each of our measures of carotenoid- and melanin-based ornamental coloration (all $p > 0.15$).

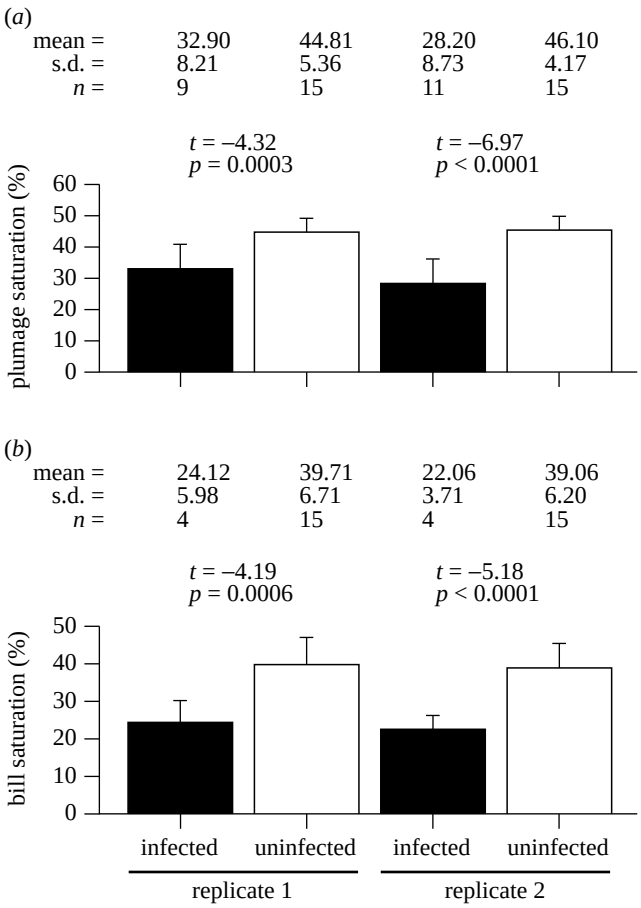

Figure 1. Effects of endoparasitism on carotenoid-based (*a*) plumage coloration and (*b*) bill coloration in captive male American goldfinches. Two replicate experiments were performed testing the same biological hypothesis. Uninfected birds (white bars) were treated for coccidial infection with a coccidiostatic drug, while infected birds (dark bars) remained untreated. Bars indicate mean ± 1 s.d.

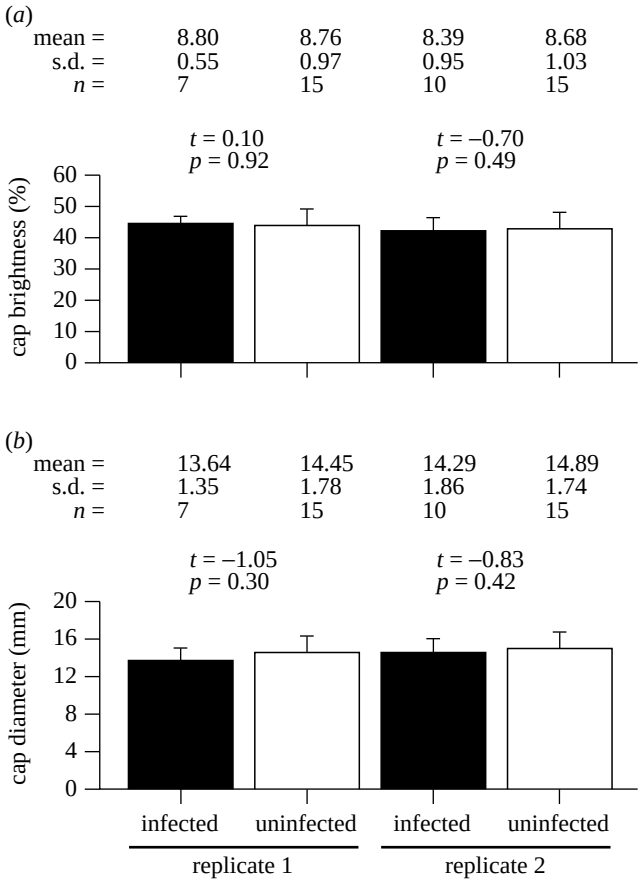

Figure 2. Effects of coccidiosis on (*a*) the brightness and (*b*) the diameter of the melanin-based ornamental cap of captive male American goldfinches. See figure 1 for description of bar charts.

3. RESULTS

(a) *Degree of endoparasitic infection*

In both replicates, males from the parasitized group were significantly more infected with isosporan coccidians than were unparasitized males (replicate 1, $U = 140$, $p < 0.0001$; replicate 2, $U = 161.5$, $p < 0.0001$; table 1), with no overlap between treatment and control groups. All but one of the males from the two infected groups shed >100 oocysts (infection scores $\geqslant 3$). In contrast, we found oocysts in faecal samples of only four males from the uninfected groups and all four of these males received infection scores of 1 (with only 3, 2, 1 and 2 oocysts detected).

(b) *Effect of parasitism on food consumption and body mass*

In both replicates, infected birds consumed significantly less food than did uninfected birds (replicate 1, $t_7 = -3.69$, $p = 0.001$; replicate 2, $t_7 = -3.43$, $p = 0.01$; table 1). Infected males weighed significantly less than did uninfected males in one of the two replicates (replicate 1, $t = -2.90$, $p = 0.003$; replicate 2, $t = -0.89$, $p = 0.24$; table 1).

(c) *Effect of parasitism on the expression of ornamental coloration*

In both replicates, carotenoid-based plumage and bill coloration of infected males was significantly less saturated than that of uninfected males (figure 1). In contrast, the brightness and diameter of the melanin-based black caps did not differ significantly between infected and uninfected males in either replicate (figure 2).

4. DISCUSSION

By comparing the effects of coccidial infection on the expression of carotenoid- and melanin-based ornamental coloration in male American goldfinches, we examined the potential for these plumage displays to reveal reliable information about the health and condition of males during moult. In accordance with Olson's (1996) study, we found that the carotenoid-based plumage and bill coloration of parasitized males was significantly less bright than the corresponding coloration developed by unparasitized males. However, neither the brightness nor the size of melanin-based black caps differed significantly between parasitized and unparasitized males in our study. The melanin ornaments grown by these two groups of males did not differ despite the fact that parasitized males also consumed significantly less food during periodic

sampling bouts and weighed significantly less than control males. Although sample sizes were small in this experiment, we had sufficient statistical power to detect the large effects of parasitism on carotenoid pigmentation ($0.5 < r^2 < 0.8$) and could have detected even smaller-effect sizes ($r^2 > 0.4$) for melanin pigmentation (Cohen 1988). Thus, our results indicate that these two ornament types are not similarly affected by parasitic infection and nutritional stress and do not convey similar information to conspecifics about the health and condition of males during moult.

These observations support one of the key assumptions of the Hamilton & Zuk (1982) hypothesis for carotenoid-based coloration but not for melanin-based ornamentation. There is a growing body of evidence indicating that parasitism negatively influences carotenoid pigmentation (e.g. Milinski & Bakker 1990; Houde & Torio 1992; Olson 1996; Thompson *et al*. 1997; Brawner *et al*. 2000). However, to our knowledge, this is the first study to consider experimentally how melanin ornamentation responds to parasitism or to any form of environmental stress. Hill & Brawner (1998) found that melanin pigmentation in the tail feathers of male house finches was unaffected by experimental infection with coccidians, but such coloration is not ornamental because house-finch tails are both drab and sexually monochromatic. Additionally, Roulin *et al*. (1998) detected little effect of environmental and nutritional conditions on the development of melanin coloration in barn owls (*Tyto alba*), but did so for the plumage of nestlings. Although little is known about the physiological requirements for melanin-pigment deposition in bird feathers, the nutritional requirements for depositing melanin are presumed to be low. Melanins are synthesized from essential amino acids that are basic dietary components (Fox 1976), and pigment deposition seems to be under strong genetic control in certain instances (Møller 1989; Norris 1993; Hudon 1997; Roulin *et al*. 1998; but see Griffith *et al*. 1999). Thus, we might expect the development of melanin coloration to be unaffected by environmental stressors such as endoparasitism and food deprivation. In support of this, J. C. Senar, J. Figuerola and J. Domenech (unpublished data) recently demonstrated that tail-feather growth, an index of nutritional condition during moult (Hill & Montgomerie 1994; Grubb 1995), was significantly associated with carotenoid but not melanin ornamentation in wild male great tits (*Parus major*).

However, other studies have suggested that ornamental melanin coloration does indeed reflect the condition of male birds during moult. Veiga & Puerta (1996) found that variation in circulating levels of blood protein was positively correlated with badge size in moulting male house sparrows (*Passer domesticus*). Otter & Ratcliffe (1999) implicated resource availability at the time of moult in the inter-seasonal population-level shift in the bib raggedness of male black-capped chickadees (*Poecile atracapillus*). Slagsvold & Lifjeld (1992) suggested that melanin coloration in male pied flycatchers (*Ficedula hypoleuca*) is condition dependent because breeding plumage coloration can be predicted by the body mass of individuals as nestlings. How do we reconcile the differences between our results and this set of observations? All of the aforementioned findings are correlational, and so the possibility remains that social factors are largely responsible for variation in melanin pigmentation and that nutritional condition is only indirectly related to aggressive ability and badge size. Even in such cases, however, individuals pigment their feathers during moult, yet display the trait and pay the associated behavioural costs at other times during the year. It seems that males would need some mechanism by which the maintenance costs incurred during either the breeding or the non-breeding season could be reliably predicted at the time the feathers are grown. The results from our experiments indicate that nutritional condition does not have any direct effect on the development of melanin ornamentation, and therefore we offer the idea that social interactions before and/or during moult may shape the degree to which ornamental melanin coloration is expressed.

Despite numerous attempts to model both verbally (e.g. Møller & Pomiankowski 1993) and game theoretically (Johnstone 1995, 1996) the adaptive function of multiple signals in animals, precisely why males are adorned with so many ornaments is still a contentious issue in evolutionary biology. Males may use 'multiple messages' to convey different information to receivers, 'redundant signals' to accurately communicate the same information, or 'unreliable signals' if certain ornaments accrue few costs and poorly communicate information (Møller & Pomiankowski 1993). Because of the differential effects of parasitism on carotenoid and melanin pigmentation in male goldfinches, our results fail to support the 'redundant signal' hypothesis. Brooks & Couldridge (1999) came to a similar conclusion after finding that the mating preferences of female guppies (*Poecilia reticulata*) independently track divergent artificial selection on melanin and carotenoid pigmentation of males. Moreover, Gray (1996) and Badyaev & Hill (2000) used phylogenetic analyses of North American passerines and cardueline finches, respectively, to show that sexual dichromatism results primarily from differences in carotenoid- and not melanin-based pigmentation, which leads to the supposition that sexual selection acts differently on the two signal types.

This study failed to demonstrate a cost of displaying melanin-based ornamentation, and so we cannot distinguish between the 'multiple message' and 'unreliable signal' hypotheses at this time. Melanin ornamentation lacks an ultraviolet signalling component in goldfinches (K. J. McGraw and G. E. Hill, unpublished data), so there is no hidden variation in the signal that may have escaped detection in this study. Nevertheless, both the extent and the brightness of melanin pigmentation varied considerably in our sample of uninfected males. Both cap diameter (coefficient of variation (CV) = 0.12) and cap brightness (CV = 0.11) were more variable than two separate measures of body size (wing chord, CV = 0.03, this study; tarsus length, CV = 0.03, Middleton 1993; test for equality of CVs, all $p < 0.0001$; Zar 1984), but neither differed in variation from carotenoid plumage saturation (CV = 0.11, both $p > 0.3$). Because melanin coloration varied even under the standardized conditions in our study, is a sexually dimorphic trait that is displayed only during the breeding season in this species, and functions as a status signal in a congener (the siskin (*Carduelis spinus*; Senar *et al*. 1993; Senar & Camerino 1998)), it is a

likely candidate for visual signalling and a careful examination of the social costs of melanin ornamentation is warranted. However, the possibility that melanin pigmentation is not costly and not a signal itself but a signal amplifier of carotenoid ornamentation also merits further consideration (Hasson 1989, 1991). Black pigmentation may function solely to improve the detectability of carotenoid coloration and/or the accuracy with which females can discriminate among potential mates (Brooks 1996).

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