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Melanin-based plumage coloration in the house finch is unaffected by coccidial infection

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For most species of birds, ornamental plumage coloration may result from two types of pigments: carotenoids and melanins. Despite the fact that melanin pigments can be synthesized by birds from basic, amino acid precursors, while carotenoids cannot be synthesized by birds and must be ingested, melanin-based plumage coloration and carotenoid-based plumage coloration have often been treated as a single trait in investigations of the function and evolution of plumage coloration. Expression of carotenoid-based coloration is known to be dependent on condition, while the effects of individual condition have not been well-tested for expression of melanin-based coloration. In this study, we experimentally tested the effect of coccidial infection of the intestinal tract of male house finches during moult on expression of melanin-based plumage coloration. Coccidial infection had a significant negative effect on carotenoid-based coloration, but it had no significant effect on melanin-based feather coloration. Unlike carotenoid-based coloration, melanin-based coloration my be cheap to produce, and honesty of melanin-based coloration may require social mediation.

Keywords: plumage coloration; sexual selection; house finch; *Carpodacus mexicanus*; coccidia; *Isospora*

1. INTRODUCTION

Since Darwin (1871) and Wallace (1895), the function of plumage coloration has intrigued evolutionary biologists. In the middle part of this century it was discovered that several different chemical and structural processes give rise to the diversity of feather colours (reviewed in Fox & Vevers 1960; Ralph 1969; Brush 1978; Vevers 1982), but until recently the possibility that colours derived through different mechanisms might serve fundamentally different functions was rarely considered by behavioural and evolutionary ecologists. With increasing interest in condition-dependent traits, and the consequent need to understand how colour expression is controlled, there has been renewed interest in the proximate control of feather coloration.

Plumage coloration results from two basic sources: (i) microstructures of the feathers that differentially reflect and absorb/scatter ambient light (i.e. structural coloration) (Auber 1957; Fox & Vevers 1960; Vevers 1982); or (ii) pigment molecules (biochromes) embedded in the feathers (Brush 1978). In most species of birds, pigment-based coloration results from either carotenoids, which produce bright red, orange, and yellow coloration, or melanins, which produce blacks, browns, greys, and earth tones (Brush 1978). Melanin pigments are synthesized by birds from the amino acids tyrosine, tryptophan, and phenylalanine (Fox 1976; Brush 1978). They are secreted by specialized melanin-producing cells (melanocytes), deposited in developing feathers, and become fixed in the keratinized feather structure (Fox 1976; Brush 1978). In contrast, carotenoids cannot be synthesized by birds, but must be ingested. Once ingested, at least some birds can modify certain carotenoid pigments (Goodwin 1984; Brush 1990) changing, for instance, yellow/orange β-carotene to red astaxanthin (Fox *et al.* 1967).

Because its expression is linked to dietary intake of scarce resources (Hill 1996*a*), carotenoid-based coloration has become a classic example of a condition-dependent trait in animals (see Andersson 1994; Hill 1995). Birds in better nutritional condition with access to larger quantities of carotenoids during moult grow redder and more intensely pigmented plumage than do birds in poor nutritional condition or with access to fewer carotenoids (Slagsvold & Lifjeld 1985; Hill 1992, 1993*a*; Hill & Montgomerie 1994; Linville & Breitwisch 1997). Moreover, birds that are infected with coccidians of the genus *Isospora* (Brawner 1997), with *Mycoplasma gallicepticum*, which causes mycoplasmal conjunctivitis (Brawner 1997), or with avian pox (Thompson *et al.* 1996), grow feathers with drabber carotenoid-based coloration than feathers grown by uninfected birds. Thus, expression of carotenoid-based coloration is highly variable, and is dependent on the condition of the bearer.

Melanin coloration has also been proposed to serve as an indicator of condition (Møller 1987*a*; Veiga & Puerta 1996). However, because melanin pigments are synthesized by birds from basic dietary components, it is not obvious how an individual's physical condition would affect expression of melanin pigmentation (Gray 1996). Moreover, most studies that have looked at melanin-based coloration have found that signal honesty must be mediated behaviourally. For instance, only dominant males that are good fighters show full expression of a

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melanin-based signal (Rohwer 1977; Møller 1987*a*,*b*), because the signal invites challenges from other males and only dominant males can back-up such a signal. Production of the signal is cheap; while wearing the signal invites challenge and is costly (Rohwer 1975, 1982).

Despite interest in the signal content of melanin-based colour displays, few studies have looked at the effects of the physical condition of birds on expression of melanin-based plumage coloration. The purpose of this study was to test experimentally the effect of a parasite on expression of melanin-based coloration. We tested the effect of intestinal coccidial parasites of the genus *Isospora* on expression of melanin-based tail coloration in the house finch (*Carpodacus mexicanus*). Isosporans infect the intestinal epithelium of their host. Depending on the species of *Isospora*, infections may be acute, lasting for only two or three weeks, or they may be chronic, lasting for several months (Boughton 1937; Box 1977; Levine 1982). Various members of the genus are widespread parasites of birds, including house finches. Coccidiosis is known to inhibit uptake of nutrients, including carotenoids, from food by the infected animal (Ruff *et al*. 1974; Augustine & Ruff 1983). We predicted that unlike expression of carotenoid-based coloration, expression of melanin-based coloration would not vary in response to infection.

2. METHODS

Details of the infection experiment can be found in Brawner (1997); here we summarize critical components of the experiment. Between 1 June and 15 July 1997, we captured approximately 100 hatching-year house finches (i.e. finches born in that calendar year) in juvenile plumage, and randomly divided them into two groups designated 'control' and 'experimental'. Only juvenile finches that had not begun their first prebasic moult were collected. Any individuals that showed symptoms (conjunctivitis) caused by *M. gallicepticum* at the time of capture, or before the start of the experiment, were released. It is not possible to sex house finches by external morphology before their first prebasic moult, but males are, on average, larger than females (Hill 1993*b*). Thus, to increase the proportion of males in our sample, we released all birds with smaller-than-average wing length.

During the experiment, birds were housed in groups of 4–10 individuals in large flight cages. Control and experimental groups were separated by at least 6 m and a solid wall, to avoid cross contamination of the control group. All birds were fed millet and black oil sunflower seed *ad libitum*. Canthaxanthin, a red carotenoid pigment, was added to the water ($0.0001\,\mathrm{g\,ml^{-1}}$) of all birds. Finches in the experiment experienced natural light cycles and temperatures, and they began and completed their first prebasic moult (involving all feathers) from late July to mid-September in synchrony with local, wild house finches (Hill 1993*b*, 1996*b*). Growth of tail feathers overlapped almost completely with growth of body plumage pigmented with carotenoids, so birds experienced the same degree of infection during carotenoid and melanin deposition.

On 5 July 1997, before any birds in our captive flocks had begun prebasic moult, each house finch in the experimental group was orally inoculated with 2000 Isosporan oocysts (for details, see Brawner 1997). On the same day, sulphadimethoxine was added to the water of males in the control group. Sulphadimethoxine is a coccidiostatic drug that halts the replication of, and oocyst production by, the parasite and stops the destruction of the intestinal epithileum that results from parasite replication. Sulphadimexothine does not necessarily kill the parasite. If the sulphadimethoxine treatment is stopped, replication and oocyst production will resume within about 7 d (W. R. Brawner III, personal observation). Thus, we were comparing birds with subclinical coccidial infection to birds with modest clinical infections.

The goal of this study was to infect one group of house finches with coccidia while maintaining the control group relatively free of coccidia. In this, we succeeded. Despite our best effort to avoid it, however, some birds also become infected with *M. gallicepticum*. Thus, various individuals were infected with only coccidia, with only *M. gallicepticum*, with both coccidia and *M. gallicepticum*, or with neither coccidia nor *M. gallicepticum*.

Once per week during the 12 weeks of the experiment, birds were removed from their flight cages and checked for conjunctivitis (one of the symptoms of *M. gallicepticum* infection). For each bird, a faecal sample was collected after 1500 h (see Brawner 1997) and placed in potassium dichromate so that coccidial oocysts could later be counted (see Brawner 1997). The number of oocysts in a faecal sample was scored on a six-point scale: 0, no oocysts observed; 1, 1–10 oocysts observed; 2, 11–100 oocysts observed; 3, 101–1000 observed; 4, 1001–10 000 oocysts observed; 5, >10 000 oocysts observed.

All individuals had completed their prebasic moult by early October, and on 7 October 1997 all birds were removed from their flight cages. Coloration of crown breast and rump feathers, and of the dorsal surface of the two central tail feathers of males were measured with a Colortron (Light Source, San Rafael, CA) reflectance spectrophotometer (see Hill (1998) for details of scoring plumage coloration with a Colortron). Three Colortron readings were taken from each of these plumage regions, and an average hue, saturation (intensity), and tone (brightness, blackness) was then recorded for each plumage region. The first principal component (PC1) from a principal components analysis of hue, saturation, and tone was used as a measure of overall plumage coloration.

3. RESULTS

Treatment of birds with sulphadimethoxine had the intended effect on captive house finches. From weeks 3–12 of the experiment, the mean coccidial score of males in the experimental group ($\bar{x}=2.73\pm0.64$) was significantly higher than the mean coccidial score of males in the control group ($\bar{x}=0.62\pm0.51$; $\chi=5.39$, $n=19$, 22, $p=0.0001$, Mann–Whitney U-test; see below for explanation of sample size), with virtually no overlap in mean coccidial scores. Thirteen males contracted *M. gallicepticum* during the experiment.

Tail colour measurements were obtained for 25 control and 19 experimental males. Of the 13 captive males that contracted *M. gallicepticum*, 10 were in the experimental group. Thus, if we eliminated all *M. gallicepticum*-infected birds from our analysis, only nine males infected with coccidia would remain, and the test between groups would have very low statistical power. Instead, we removed the three control males infected with *M. gallicepticum* from the analysis, leaving two groups: (i) 22 control males with neither coccidial infection nor *M. gallicepticum* infection; and (ii) 19 experimental males with coccidial

Table 1. *Mean (±s.d.) tristimulus colour scores for melanin-based tail coloration and carotenoid-based feather coloration of male house finches that were infected with coccidia or that were maintained with subclinical coccidial infections*

feathered region	type of pigmentation	colour parameter[b]	control[c] ($n=22$)	experimental[d] ($n=19$)	Z[e]	p
tail	melanin	hue	21.64±4.51	22.63±3.95	−0.64	0.53
		saturation	19.62±4.75	18.52±3.71	−106	0.29
		tone	20.30±3.39	19.30±2.96	−0.84	0.40
		PC1	−0.14±1.16	−0.05±0.81	−0.17	0.87
breast	carotenoid[a]	hue	10.09±1.84	13.88±2.19	−4.49	0.0001
		saturation	51.33±3.72	46.47±5.49	−2.81	0.005
		tone	48.27±4.08	46.62±3.66	−1.16	0.25
		PC1	−0.52±0.83	0.65±0.96	−2.69	0.007

[a]See Brawner (1997) for details of effects of parasites on carotenoid-based pigmentation.
[b]PC1 combines hue, saturation, and tone. See text for details.
[c]Control birds have no *M. gallicepticum* infection and coccidial infection reduced to subclinical level by treatment with sulphadimethoxine.
[d]Experimental birds were infected with coccidia, and eight of these birds also were infected with *M. gallicepticum*.
[e]Mann–Whitney *U*-test.

infection, 8 of which also had *M. gallicepticum* infection. Because we predicted no effect of treatment on melanin pigmentation, this constituted the most conservative test of our hypothesis. Retaining *M. gallicepticum*-infected males in our experimental group maximized the power of our test, and the addition of a secondary infection in some males of the experimental group would be expected to further negatively impact on the expression of plumage coloration.

As reported by Brawner (1997), the hue of carotenoid-based coloration was significantly impacted by both coccidial infection and *M. gallicepticum* infection. Plumage of males infected with either or both of these parasites was less red and less saturated than the plumage of control males (table l; for details, see Brawner 1997). In contrast, the mean hue, saturation, and tone of melanin-based tail coloration of males infected with coccidia, or with both coccidia and *M. gallicepticum*, were virtually identical to, and not significantly different from, control males that had neither disease (table l).

4. DISCUSSION

In this study, and in a companion study (Brawner 1997), we tested the effect of parasite infection on expression of both carotenoid-based feather coloration and melanin-based feather coloration in male house finches. Carotenoid pigmentation was significantly negatively affected by both infection with *Isospora* and infection with *M. gallicepticum*. In contrast, in the same males whose carotenoid pigmentation was significantly affected by parasitism, the melanin-based pigmentation of tail feathers was not affected by the infection; in other words, the coloration of tail feathers of males that were sick during moult did not differ statistically in hue, saturation, tone, or total plumage coloration from the coloration of tail feathers of males that were not sick. To our knowledge, this is the first time that the effect of parasitism on melanin-based plumage coloration has been tested experimentally in songbirds.

In another study of the effect of parasites on expression of melanin-based coloration, Zuk *et al.* (1990) looked at the effect of nematode infection on a variety of traits, including melanin pigmentation of feathers, in red jungle fowl (*Gallus gallus*). They reported a change in the melanin-based coloration of the hackle feathers and saddle feathers of infected males, but because the results were presented in a complex, multivariate analysis, in which statistical tests were performed only on composite variables that included both melanin pigmentation and other traits, it is not possible to assess how large was the effect on melanin pigmentation alone. In a study of the effects of nutrition on melanin pigmentation, Veiga & Puerta (1996) found that male house sparrows that were nutritionally stressed grew smaller patches of melanin-based black coloration than males that were fed *ad libitum*. The authors did not note any effect on the blackness of throat feathers. Thus, the number of ventral feathers of house sparrows that had black pigmentation was affected by diet, but the blackness of the patch was not affected. More research on dietary and condition effects on melanin pigmentation are needed, but from published accounts it appears that birds adjust the size of melanin-based patches based on their condition, and that this decision is little affected by the ability to produce melanin.

Although these are the only published, experimental studies testing the effect of individual condition on expression of melanin-based feather coloration, substantial anecdotal evidence supports our finding that carotenoid-based coloration is more vulnerable to the effects of diet, parasitism, and overall condition than is melanin-based coloration. It is common in the zoological and avicultural literature to find articles on how to avoid loss of, or change in, expression of carotenoid-based plumage coloration in captive birds (e.g. Delacour 1928; Bruning 1971), but loss of, or change in, melanin-based plumage coloration is not discussed. This suggests that birds grow feathers with normal melanin pigmentation under a variety of captive conditions. The fact that no previous studies have reported this apparent tolerance of melanin pigmentation to environmental stresses probably reflects a general attitude among zoobiologists that such observations are too trivial to publish. However, documentation of such resistance to

change in expression of melanin-based plumage pigmentation is obviously not trivial to evolutionary biologists interested in the evolution of plumage signals.

One shortcoming of our study is that we focused on melanin pigmentation that was not ornamental. In general, sexually selected traits are thought to be less developmentally stable than traits that have arisen and are maintained by natural selection (Møller 1992; Møller & Höglund 1991; Møller & Pomiankowski 1993; Hill 1995). Thus, the most appropriate test of the idea that carotenoid pigmentation is inherently more responsive to environmental effects than melanin pigmentation is to look at the effect of parasites or diet on a carotenoid-based feather ornament and on a melanin-based feather ornament simultaneously in the same experiment. We encourage further testing of the effects of condition on expression of melanin-based ornaments with this sort of study.

Melanin and carotenoid pigments are the two most common and widespread pigments used by birds to colour feathers. Both types of pigments are deposited in feathers of some bird species in a manner that makes them appear ornamental. Because they both contribute to the showiness of plumage, melanin-based plumage coloration and carotenoid-based plumage coloration are often treated as a single form of display, with the implicit assumption that insight into one form of coloration can be extrapolated to other types of coloration. However, more detailed studies of the proximate control of melanin and carotenoid pigmentation are revealing that there are fundamental differences in the proximate control of these two types of pigmentation, and that these differences in proximate control suggest that they function as distinctly different forms of visual signals (see Gray 1996).

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