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EFFECTS OF COCCIDIAL AND MYCOPLASMAL INFECTIONS ON CAROTENOID-BASED PLUMAGE PIGMENTATION IN MALE HOUSE FINCHES

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ABSTRACT.—Carotenoid pigments produce the ornamental red, orange, and yellow integumentary coloration of many species of animals. Among individuals of a population, the hue and saturation of carotenoid-based ornaments can be extremely variable, and studies of fish and birds have shown that females generally prefer males that display the most saturated and reddest coloration. Consequently, there has been a great deal of interest in determining the proximate factors that affect individual expression of carotenoid-based pigmentation. Parasites might affect production of ornamental coloration, and the Hamilton-Zuk hypothesis proposes that parasitized males will show decreased expression of the secondary sexual traits preferred by females. We found that captive male House Finches (*Carpodacus mexicanus*) experimentally infected with *Isospora* spp. (coccidians) and / or *Mycoplasma gallisepticum* produced carotenoid-based plumage coloration that was significantly less red and less saturated than that of noninfected males. These observations validate a necessary condition of the Hamilton-Zuk hypothesis, but heritable resistance to the pathogens we examined remains to be demonstrated. *Received 6 July 1999, accepted 27 April 2000.*

HAMILTON AND ZUK (1982) proposed that secondary sexual characteristics evolved because they serve as reliable indicators of heritable resistance to parasites and disease. According to their hypothesis, only resistant males with low parasite loads can produce the most exaggerated and costly sexual ornamentation. Males with high parasite loads show reduced expression of their sexual ornaments. Consequently, by choosing to mate with a highly ornamented male, a female receives benefits for her offspring in the form of genes for parasite resistance.

The Hamilton-Zuk hypothesis makes two predictions concerning the relationship between parasitic infections and secondary sexual characteristics: (1) species that are most subject to infection should have more developed and/or conspicuous secondary sexual traits (brighter coloration, more elaborate plumage, etc.); (2) within species, preferred mates should be the least parasitized. Here, we present a test of the second, or intraspecific, prediction of the hypothesis.

For the Hamilton-Zuk hypothesis to work within a species, three critical predictions must be met: (1) the degree of development of secondary sexual characteristics should be related to the intensity of parasitic infection; (2) females should base their choice of mates on expression of the ornamental trait that is correlated with parasitism; and (3) resistance to the parasite must be heritable (McLennan and Brooks 1991). Møller (1988, 1990) has provided the best support for the hypothesis by finding that each of these requirements was satisfied in a host-parasite relationship involving Barn Swallows (*Hirundo rustica*) and hematophagous mites (*Ornithonyssus bursa*). Other studies have shown that at least two of the three requirements are met in a variety of host-parasite relationships (see Hamilton and Poulin 1997).

One of the most widespread ornamental traits in animals is carotenoid-based coloration of the integument. Animals cannot produce carotenoids and must acquire these pigments in their diet (Goodwin 1984). Because carotenoids must be obtained from the environment, carotenoid pigmentation is likely to be affected by environmental factors such as parasites; indeed, the intensity of infection by various parasites is negatively related to display of carotenoid-based ornamental coloration in some birds and fishes (e.g. Milinski and Bakker 1990, Houde and Torio 1992, Thompson et al. 1997, Merilä et al. 1999).

No specific mechanism has been proposed

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for how a parasitic infection depresses the expression of carotenoid pigmentation. According to Brush (1978), carotenoid pigmentation involves four main steps: absorption, transport, metabolism, and deposition. The process of absorption may be particularly susceptible to the effects of parasitic infection. Several intestinal parasites have been shown (either by direct measurement of carotenoid levels in serum and tissue, or by observation of carotenoid-based ornaments) to reduce absorption of dietary carotenoids (Ruff et al. 1974; Allen 1986, 1992; Tyczkowski et al. 1991). The conversion of yellow pigments to red pigments, as well as other processes involved in the metabolism of carotenoids, appears to be energetically costly (Hill 1996, 2000). Individuals that expend energy fighting parasite infestations may not be able to allocate as much energy to the metabolism or transport of pigments used in sexual displays (see Hill 2000). Infection also may lead to decreased energy expenditure on foraging so that fewer carotenoids are ingested.

The initial objective of our study was to assess the effects of coccidial infections on the carotenoid-based plumage pigmentation of male House Finches (*Carpodacus mexicanus*). Because of an epidemic of *Mycoplasma gallisepticum* among House Finches (Nolan et al. 1998), *M. gallisepticum* was inadvertently introduced into the experimental groups in both years of the study. This also allowed us to assess the effects of mycoplasmosis (which causes severe conjunctivitis in House Finches) on expression of carotenoid-based plumage coloration. We predicted that male finches with coccidial and/or mycoplasmal infections would show decreased plumage redness compared with uninfected males.

Methods

Study species.—The House Finch is a socially monogamous, sexually dichromatic species. Female plumage is grayish brown with streaking on the breast. Males have similar plumage coloration but with ornamental carotenoid pigmentation on their crown, breast, and rump. The degree of pigmentation is variable and ranges from dull yellow through orange to bright red (Hill 1992, 1993). Plumage redness and color intensity are a function of the type and quantity of carotenoid pigments present in the feathers (Inouye 1999). Males with a higher proportion of red (vs. yellow) pigments and higher pigment concentration have redder and more brightly colored plumage (Inouye 1999). House Finches undergo one complete molt annually in late summer or early fall. Experimental studies have shown that female House Finches prefer as mates the reddest and most intensely colored males available to them (Hill 1990, 1991, 1994a; Hill et al. 1999).

Coccidians of the genus *Isospora* are common parasites of House Finches. They are protozoans that generally are thought of as intestinal parasites, and they exhibit both sexual and asexual phases. The sexual phase results in the production of oocysts and occurs in the intestinal epithelium of the host. Asexual stages are also found in the intestinal epithelium; however, in some species the asexual stages are thought to disseminate to other organs (liver and spleen) before returning to the intestine for sexual replication. Isosporans are transmitted by a fecal-oral route. Infection occurs when an oocyst, shed in the feces of a host, is ingested by another host.

Mycoplasmal conjunctivitis was first observed in the eastern population of House Finches in February 1994, in the Washington, D.C. area (Fischer et al. 1997). Infected birds are easily recognized after the appearance of clinical signs of disease, although House Finches may be infected without showing clinical signs (Luttrell et al. 1996). In the early stages of clinical disease, the eyelids and conjunctiva are only slightly reddened and are not very swollen. As the infection progresses, the lids eventually become swollen shut. A purulent discharge from the eyes forms a crusty layer around and eventually over the swollen eyelids. A nasal discharge also may be present. Infection may result in unilateral or bilateral conjunctivitis.

The causative agent of this disease has been identified as *Mycoplasma gallisepticum* (Ley et al. 1996), which is a well-known pathogen of poultry that causes chronic respiratory disease in chickens and infectious sinusitis in turkeys. This parasite apparently "jumped" from poultry, presumably chickens, in the mid-Atlantic region of the United States in 1994; House Finches (and other passerines) almost certainly had not been exposed to the pathogen before this colonization event. Mycoplasmas can be transmitted horizontally by direct contact, moisture droplets, and dust and vertically through the eggs (Yoder 1991). In adult House Finches, transmission likely occurs by direct contact during courtship feeding or at crowded feeders. Infection through moisture droplets also may play a role at heavily used feeders. Nestlings with infected parents are likely to become infected. If the disease is not passed vertically to offspring before hatching, it almost certainly would be passed to them in the nest after hatching.

Collection of host and parasite.—We tested the effect of parasite infection on male House Finches that were growing their first basic plumage via their first prebasic molt. Hatching-year (HY) House Finches (individuals born in the calendar year of the study)

molt from mid-July through September, after which their plumage coloration is fixed until the next complete molt a year later.

The experiment was conducted in the summer and fall of 1996 and again in 1997. All birds were captured in Lee County, Alabama. The sex of HY House Finches cannot be determined by visual inspection, so approximately half of the birds that we captured and held through molt were females and were not used in comparisons. Throughout this paper, sample size refers only to males. In 1996, 13 HY males were captured in July or August. Some of these birds had initiated molt, although no birds had grown more than about 10% of their body plumage. In 1997, 51 HY males were captured at least three weeks before they had started molt. Finches were held in outdoor flight cages (3.7 × 1.5 × 2.4 m) with sealed concrete floors. Birds were given a minimum of one week to adjust to captivity before the beginning of an experiment.

We measured wing length of captured birds in both years and weighed each bird in 1997 only. We also recorded presence or absence of avian pox and conjunctivitis. Birds showing signs of pox (lesions on feet) or conjunctivitis (reddened, swollen conjunctiva) were released. Each bird was banded with a numbered aluminum leg band. We recorded the number of feather mites on the primary feathers on a five-point scale developed by Thompson et al. (1997:273): 0 (no mites), 1 (occasional mites), 2 (many mites along rachis of some feathers), 3 (nearly every feather with hundreds of mites), and 4 (all feathers with hundreds to thousands of mites). Separate molt scores were assigned to each of six regions on the bird: wings, throat, breast, belly, crown, and back (Rohwer 1989).

We collected isosporan oocysts from eight naturally infected adult House Finches in 1996 and from 20 naturally infected adult and HY finches in 1997. These birds were housed individually in small (26.7 × 21.6 × 34.3 cm) cages with mesh bottoms. An aluminum pan lined with wet paper towels was placed beneath each cage to collect feces. The feces were removed from the pans every 12 h and placed in 2.2% potassium dichromate solution. A thin layer of this solution containing feces was exposed to air for five days to allow oocysts to sporulate. After two weeks of collection and sporulation, oocysts were removed from the feces by flotation in Sheather's solution. Oocysts were then placed in fresh potassium dichromate and stored at 4°C for later use. Four days before inoculation, oocysts were washed with distilled water to remove the potassium dichromate solution and placed in Hanks' balanced salt solution (HBSS). A small amount of penicillin and streptomycin were added to prevent bacterial growth. A hemacytometer, which allows one to determine the density of evenly suspended particles in solution, was used to count viable, fully sporulated oocysts.

To identity the coccidia infecting House Finches in the local population, 25 oocysts, taken from a mix of the oocysts collected from wild House Finches as described above, were examined using a Nomarski interference contrast microscope. We recorded the length and width of the oocysts and sporocysts and noted presence or absence of steida and sub-steida bodies as well as the shape and color of polar granules. This information was then compared with characteristics of previously described coccidians.

Experimental treatment.—We tested possible anticoccidial drugs for effectiveness against isosporan infections in House Finches using known infected birds in the summer of 1996. Amprolium was ineffective in reducing coccidial infection in House Finches, and pyrimethamine, which is temperature and light sensitive, was effective only indoors. On the other hand, sulfadimethoxine reduced coccidial infection in House Finches to subclinical levels when added to the drinking water (0.496 mg per mL) of birds in outdoor cages. During treatment with sulfadimethoxine, oocysts stopped appearing in the feces of birds, indicating that parasitic replication (the pathogenic phase of the life cycle) had been halted. However, when treatment stopped, oocyst production resumed within several days. Thus, the action of sulfadimethoxine in House Finches at 0.496 mg mL severely depressed but did not eliminate coccidial infection (e.g. it was coccidiostatic but not coccidiocidal).

We used sulfadimethoxine to create "uninfected" groups of finches for our experiments, but what our study really tested was the color of feathers grown by finches with subclinical versus clinical coccidial infections. Sulfadimethoxine is widely used in the poultry industry as a coccidiostatic drug. For poultry farmers, the carotenoid-based coloration of the fat, skin, and egg yolks is critical to the commercial viability of the product. Moreover, an extensive search of the literature as well as discussions with poultry experts indicated no evidence that sulfadimethoxine affects carotenoid pigmentation. Therefore, we assumed that sulfadimethoxine had no effect on carotenoid pigmentation of House Finches.

In 1996, both control and experimental birds were treated with sulfadimethoxine and pyrimethamine for six days prior to the start of the experiment in an unsuccessful attempt to completely eliminate coccidial infection in both groups. In 1997, no drugs were administered before the start of the experiment. In 1996, HY males were randomly divided into two treatment groups of six and seven birds; in 1997, HY birds were randomly divided into two treatment groups of 25 and 26 birds. In 1996, all control birds were housed together in one cage and all treatment birds were housed together in one cage. In 1997, the 25 control males were housed in two cages with 12 and 13 males per cage, and the 26 treatment males were housed in two cages with 13 males per cage. In

each year, males in the treatment group were inoculated with 2,000 isosporan oocysts per bird, and birds in the control group were inoculated with saline solution. Previously, it was determined that 2,000 oocysts establish a moderate infection that is not fatal (W. R. Brawner unpubl. data). Inoculation was accomplished by sliding a 20-gauge needle with a bulbous end (feeding needle) down the esophagus of the bird and injecting 0.2 mL HBSS that contained 2,000 oocysts. In both years, the second group was treated with sulfadimethoxine (0.496 mg per mL in drinking water) for the duration of the experiment.

Cages housing control groups were separated by a solid wall from cages housing inoculated groups. Each cage was equipped with a food dish, water bowl, and a bowl of grit. Water was changed and food dishes were filled daily, and cages were cleaned weekly with a high-pressure washer. All birds were fed a diet of millet and sunflower seeds. Canthaxanthin, a carotenoid pigment that occurs in the plumage of wild House Finches (Inouye 1999) and that has been shown to produce red coloration in captive House Finches (Brush and Power 1976; Hill 1992, 1993), was added to the drinking water of all birds at a concentration of 10 mg per L in 1996 and 125 mg per L in 1997. Both of these doses of canthaxanthin are substantially lower than the doses used in previous feeding experiments on House Finches (Hill 1992, 1993). Our goal was to provide House Finches with a modest amount of dietary carotenoids. Under the dose used in 1997, no males attained more than dull orange plumage, so we increased the concentration of carotenoids added to water in 1998.

In both years, attempts to prevent *M. gallisepticum* infection of experimental birds by removing birds with clinical signs of infection before the start of the experiment were not successful. No birds began the experiment with clinical signs of disease, but in all cages several birds developed conjunctivitis during the study. Both groups were treated for mycoplasmosis with tylosin tartrate; birds were treated twice during the 10-week experiment in 1996 and were treated for three-day periods with seven-day breaks between treatments in 1997. The drug was added to the drinking water at a concentration of 0.65 mL per L. Each treatment period consisted of three consecutive days. Tylosin tartrate does not completely cure finches of *M. gallisepticum* infection, but it does decrease severity and prevent death. During treatment with tylosin tartrate, administration of sulfadimethoxine to the drug-treated group was discontinued. Sulfadimethoxine treatment was resumed after treatment for *M. gallisepticum* infection was completed.

Fecal samples were collected from all birds within one week of inoculation and at the end of the study. Fecal samples were collected twice during the study in 1996 and were taken weekly throughout the study in 1997. All fecal samples were taken between 1500 and 2100. Afternoon sampling was necessary because, like many passerines, House Finches infected with *Isospora* show a diurnal periodicity with peak oocyst elimination in late afternoon (Brawner and Hill 1999).

Feces collected from each bird were placed in a glass vial with approximately 2 mL of 2.2% potassium dichromate and broken into small pieces using a small wooden dowel. The content of each vial was pipetted into a 15-mL centrifuge tube, and Sheather's solution was added until a meniscus formed at the top of the tube. We placed a coverslip on top of each tube and then centrifuged the tubes for 7 to 8 min at 2,100 rpm. Coverslips were then removed, placed on glass slides, and examined for oocysts using a light microscope. The number of oocysts present on each coverslip was recorded on a scale from 0 to 5: 0 = no oocysts, 1 = 1 to 10 oocysts, 2 = 11 to 100 oocysts, 3 = 101 to 1,000 oocysts, 4 = 1,001 to 10,000 oocysts, and 5 = more than 10,000 oocysts. All birds were checked weekly for signs of conjunctivitis.

We scored hue, saturation, and brightness of incoming plumage using a Colortron reflectance spectrophotometer (see Hill 1998). The Colortron scores saturation and brightness as percent light reflected and percent black in color, respectively, so higher scores correspond to more intense and blacker colors. Hue scores are unitless values on a 360-degree color wheel in which redder hues score lower than orange and yellow hues. Thus, bright red plumage would have a high saturation and brightness score but a low hue score compared with drab yellow plumage.

Statistical analysis.—We used Wilcoxon rank-sum tests for comparisons of pre-experimental scores and for all comparisons of hue, saturation, and brightness scores between various groups of birds. A Wilcoxon signed-rank test was used for comparing mite infestation within groups before and after molt. One-tailed tests were used when we had a clear *a priori* prediction for the direction of an effect (e.g. parasites reduce the redness and brightness of plumage). All *P*-values are two-tailed unless otherwise noted.

Results

Identification of coccidians and test of anti-coccidial drugs.—All 25 oocysts measured were typical of the genus *Isospora* and contained two sporocysts with four sporozoites in each sporocyst. Measurements taken from 10 of the 25 oocysts fell within the ranges given by Anwar (1966) and Box (1975) for *I. lacazei*. The oocysts also fit the descriptions of *I. lacazei* given by these authors with respect to number and shape of polar granules and presence or absence of steida and sub-steida bodies. The remaining 15 oocysts did not fit descriptions of *I.*

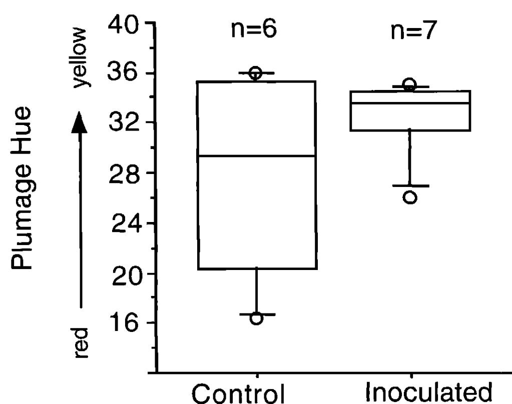

FIG. 1. Box plots of plumage hue of male House Finches inoculated with 2,000 *Isospora* oocysts or maintained on coccidia-suppressing medication (control) during molt in 1996. Horizontal bars denote 10th, 25th, 50th, 75th, and 90th percentiles; circles are for individuals outside this range.

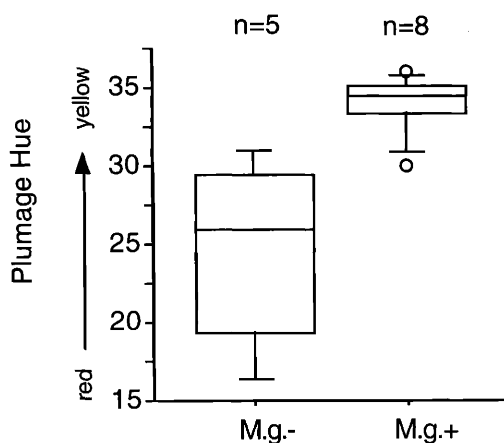

FIG. 2. Box plots of plumage hue of male House Finches infected (M.g.+) or not infected (M.g.−) with *Mycoplasma gallicepticum* during molt in 1996 (see Fig. 1 for description of plots).

lacazei, but we could not identify them to species. Thus, in this experiment, birds were infected with at least two species of *Isospora* (*I. lacazei* and an unidentified species).

Treatment of birds with sulfadimethoxine had the intended effect on captive House Finches. From weeks 3 to 13 of the experiment in 1997, the mean coccidial score of 26 males in the experimental group ($\bar{x}$ = 2.74 ± SD of 0.92) was significantly higher than that of 25 males in the control group ($\bar{x}$ = 0.61 ± 0.47; one-tailed Mann-Whitney *U*-test, Z = 5.70, P = 0.0001), with no overlap in the individual coccidial scores of males in the two groups.

Effects of coccidiosis and mycoplasmosis on plumage color in 1996.—At capture, inoculated and control groups did not differ in wing length (Z = 0.00, P = 1.00) or feather mite scores (Z = −0.71, P = 0.48), and no birds had pox lesions. Five of six males in the 1996 control group had not started molting when the experiment was initiated. The sixth bird had molt scores of 1 for throat, breast, belly, and back and a score of 0 for the crown and was replacing one primary. Five of seven males in the 1996 inoculated group had not started molting at the time of capture. The sixth bird had molt scores of 0 for all areas except the throat, which had a score of 1. The seventh bird scored 0 for the throat, belly, crown, and wings; 1 for the back; and 2 for the breast.

After they had completed molt, birds in the 1996 inoculated group were less red than birds in the 1996 control group, but the difference was not significant (one-tailed Z = −0.64, P = 0.26; Fig. 1). Control and inoculated males did not differ significantly in plumage saturation or brightness ($P > 0.5$, one-tailed test for both comparisons). Males without conjunctivitis (Mg−) were significantly redder (Z = −2.71, P = 0.003; Fig. 2), more saturated (Z = −2.20, P = 0.03), and brighter (Z = −2.64, P = 0.01) than those with conjunctivitis (Mg+), regardless of coccidial infection status (all tests one-tailed).

Because some birds were simultaneously infected with coccidia and *M. gallisepticum*, we looked for an interaction between the separate effects of the two diseases on plumage color. To assess the effects of coccidial infection independent of mycoplasmal infections, all birds that had conjunctivitis were removed from the analysis. This left three birds in the 1996 control group with neither coccidial nor mycoplasmal infection (1996 control Mg−) and two birds in the 1996 inoculated group with coccidial but not mycoplasmal infections (1996 inoculated Mg−; Fig. 3). Owing to small sample sizes, statistical analysis was not performed on these groups.

To assess the effects of mycoplasmal infection independent of coccidial infection, hue scores for the three 1996 control birds without conjunctivitis (1996 control Mg−) were compared with those for the three 1996 control birds with conjunctivitis (1996 control Mg+).

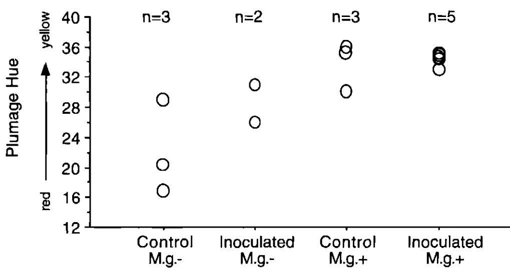

FIG. 3. Plumage hues of male House Finches with no coccidiosis or mycoplasmosis (control M.g.−), with only coccidiosis (inoculated M.g.−), with only mycoplasmosis (control M.g.+), or with coccidiosis and mycoplasmosis (inoculated M.g.+) during molt in 1996. All data points are shown.

None of these birds was inoculated with isosporan oocysts. Even with this small sample size, the hue of birds with conjunctivitis was significantly higher than that of birds without conjunctivitis (one-tailed $Z = 1.75$, $P = 0.04$; Fig. 3).

To assess the combined effects of the two diseases, hue scores of the five birds with both infections (1996 inoculated Mg+) were compared with those of the three birds with neither infection (1996 control Mg−). Again, despite very small sample sizes, the hue of birds with both infections was significantly higher than that of birds with neither infection ($Z = -2.08$, $P = 0.02$; Fig. 3).

Effect of coccidiosis and mycoplasmosis on plumage color in 1997.—At capture, inoculated and control groups in 1997 did not differ in body mass ($Z = -0.47$, $P = 0.64$) or mite scores ($Z = 0.15$, $P = 0.89$). No birds in either group had initiated molt prior to the inoculation date.

After they had molted, the mean hue of the 1997 control group was significantly lower than that of the 1997 inoculated group (one-tailed $Z = -5.22$, $P < 0.0001$; Fig. 4), indicating that the control group produced significantly redder plumage. Color saturation (one-tailed $Z = 3.92$, $P < 0.0001$) and brightness (one-tailed $Z = 2.20$, $P = 0.01$) were significantly higher in the 1997 control group than in the 1997 inoculated group (Fig. 4). The mean hue, saturation, and brightness scores were calculated for birds with and without conjunctivitis, regardless of their coccidial infection status. The hue of birds without conjunctivitis was significantly lower than that of birds with the disease (one-tailed $Z = 4.58$, $P < 0.0001$; Fig.5), and saturation

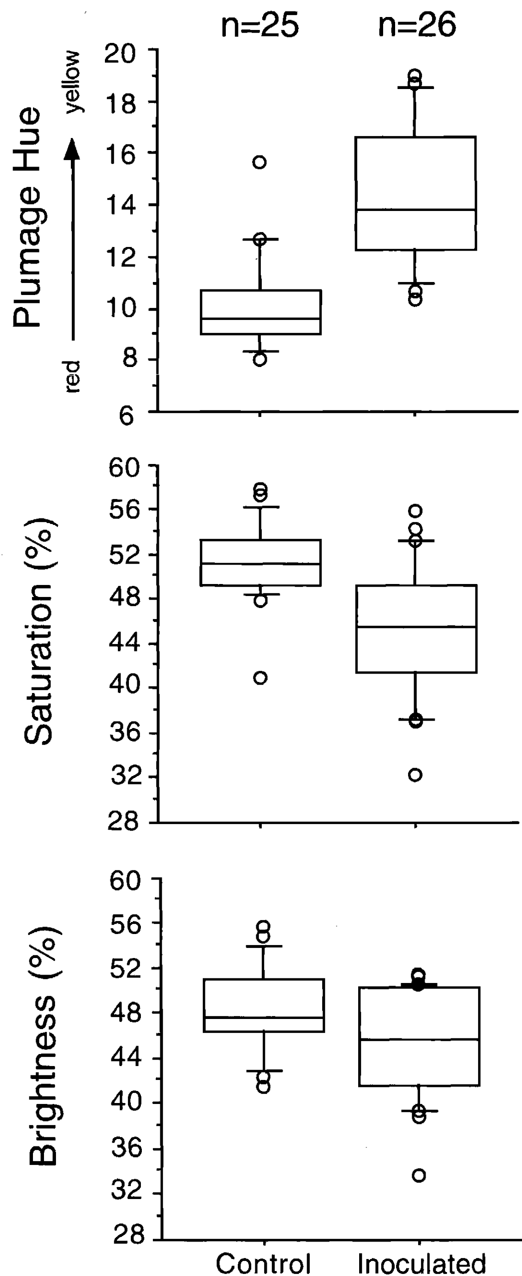

FIG. 4. Box plots of hue, saturation, and brightness of carotenoid-based plumage of male House Finches inoculated with 2,000 *Isospora* oocysts or maintained on coccidia-suppressing medication (control) during molt in 1997 (see Fig. 1 for description of plots).

(one-tailed $Z = -3.39$, $P = 0.0004$) and brightness ($Z = -3.27$, $P = 0.0006$; one-tailed test) were significantly higher in the group without conjunctivitis (Fig. 5).

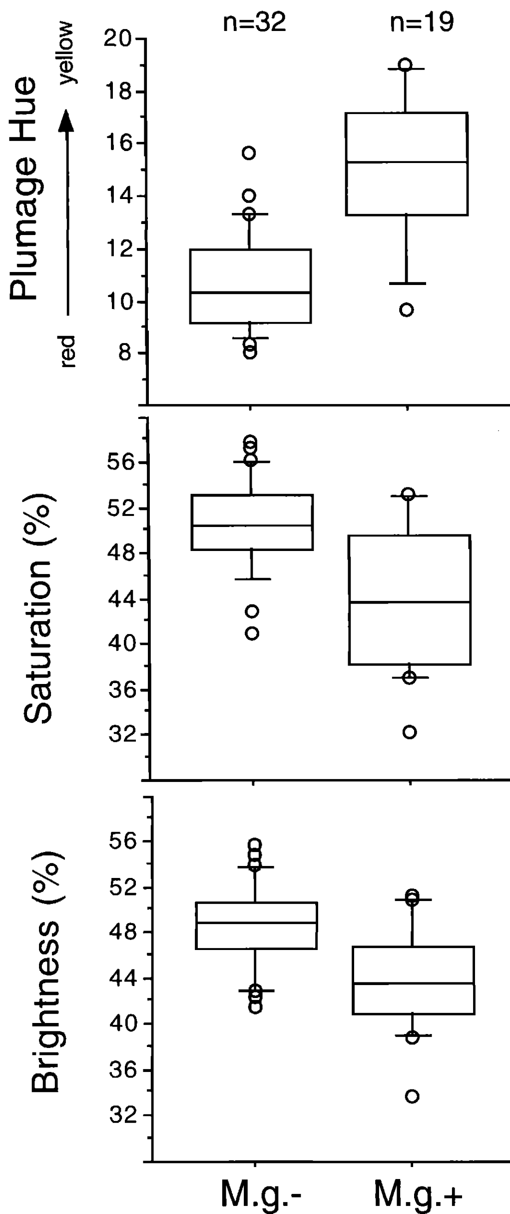

FIG. 5. Box plots of hue, saturation, and brightness of carotenoid-based plumage of male House Finches infected (M.g.+) or not infected (M.g.−) with *Mycoplasma gallicepticum* during molt in 1997 (see Fig. 1 for description of plots).

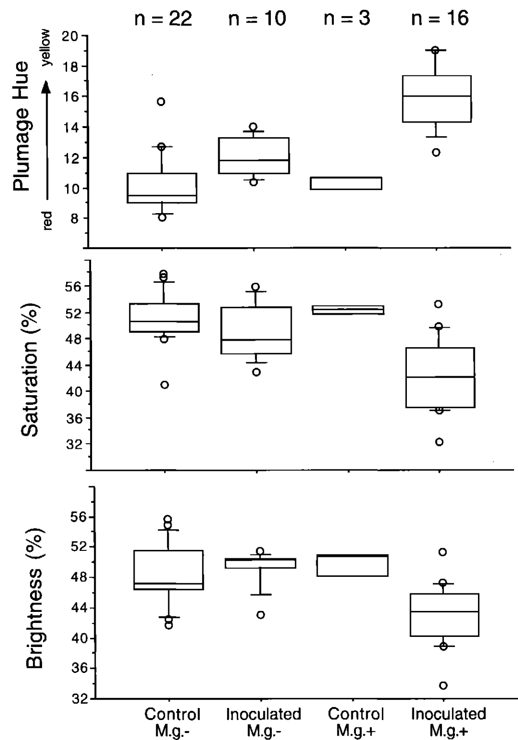

FIG. 6. Box plots of hue, saturation, and brightness of carotenoid-based plumage of male House Finches with no coccidiosis or mycoplasmosis (control M.g.−), with only coccidiosis (inoculated M.g.+), with only mycoplasmosis (control M.g.+), or with coccidiosis and mycoplasmosis (inoculated M.g.+) during molt in 1997 (see Fig. 1 for description of plots).

To assess the effects of coccidiosis independent of mycoplasmal infection, all birds with conjunctivitis were removed from the analysis. The mean hue of the 1997 control group remained significantly lower than that of the 1997 inoculated group after the removal of birds with conjunctivitis (one-tailed $Z = 2.99$, $P = 0.001$; Fig. 6). Saturation (one-tailed $Z = -1.63$, $P = 0.05$) and brightness (one-tailed $Z = 1.02$, $P = 0.15$) were not significantly different after the removal of the birds with conjunctivitis (Fig. 6).

To assess the effects of mycoplasmal infection on coloration independent of coccidial infection, we compared scores of birds with (1997 control Mg+) and without (1997 control Mg−) conjunctivitis in the control group (not inoculated, treated with sulfadimethoxine). We found no significant differences in hue ($Z = 0.88$, $P = 0.19$), saturation ($Z = 0.79$, $P = 0.22$), or brightness ($Z = 0.67$, $P = 0.25$) between the two groups (all tests one-tailed; Fig. 6).

To assess the combined effects of the two diseases, we compared scores from the group of birds with both infections (1997 inoculated

Mg+) with scores from the birds with neither infection (1997 control Mg−). The mean hue of the group with neither infection was significantly lower than that of the group with both infections ($Z = 4.93$, $P < 0.0001$; Fig. 6), and the mean saturation ($Z = -4.11$, $P < 0.0001$) and brightness ($Z = -3.47$, $P = 0.0005$) were significantly higher in the group with neither infection (all tests one-tailed; Fig. 6).

Discussion

We found a significant effect of the parasites *Isospora* spp. and *M. gallisepticum* on the expression of carotenoid-based plumage coloration of male House Finches. The effect of coccidia on plumage was most striking in the 1997 experiment, but the 1996 experiment was confounded by a very small sample size and the late date (after the start of molt) on which subjects were captured. In contrast, the effect of *M. gallisepticum* on plumage coloration was most evident in 1996. By 1997, we had developed a much better regimen for treating birds that were infected by *M. gallisepticum*; as a result, birds that contracted *M. gallisepticum* suffered from shorter and less severe infections in 1997 than in 1996. Despite these complicating factors, our experiments demonstrated that both parasites negatively influenced carotenoid-based plumage coloration of male House Finches that are infected during molt.

Our study extends observations that have been made on the effects of eimerian coccidia on plasma carotenoids and integumentary coloration in poultry (Allen 1987, 1992; Ruff et al. 1974; Tyczkowski et al. 1991). Although evolutionary biologists have speculated that coccidial infection should affect development of plumage coloration in passerines (Zuk 1992), no previous studies have shown such an effect. McLennan and Brooks (1991) warned against making generalizations from one host-parasite relationship to another, and although eimerians and isosporans follow similar life cycles, they exhibit some important differences. For example, eimerians of chickens complete all phases of replication in the intestine of their host, whereas isosporans have been reported to have extra-intestinal stages (Box 1981, Sironi 1994, Giacomo et al. 1997). *Isospora* species such as *I. lacazei* (Sironi 1994) and *I. serini* (Box 1981) that produce extra-intestinal infection seem to produce chronic infections. The patent period (time from first appearance of oocysts in feces until no oocysts appear in feces) for these chronic infections is measured in months (Anwar 1966, Box 1981) versus days or weeks for the patent period of species without extra-intestinal stages. This is important because chronic infections are predicted to work best in a Hamilton-Zuk process (Hamilton and Zuk 1982).

Isosporans generally are considered to be less pathogenic than eimerians; however, isosporosis has been reported to cause high mortality in some passerines such as European Goldfinches (*Carduelis carduelis*; Sironi 1994) and Black Siskins (*C. atrata*; Giacomo et al. 1997). These reports show that isosporosis can be very pathogenic in some passerines and can cause the types of intestinal pathology that have been associated with reduced carotenoid absorption in poultry.

Our results suggest that isosporan infection in House Finches is pathogenic enough to cause decreased pigmentation of feathers with carotenoids. Allen (1986, 1987, 1992) suggested that decreased carotenoid absorption from damaged intestinal epithelium was a mechanism for reduced carotenoid pigmentation associated with coccidiosis. Despite its effect on carotenoid-based pigmentation, coccidial infection did not impair finches in the inoculated group to the point that they behaved different from control birds or grew feathers at a grossly different rate (we did not test for subtle differences in feather growth rate). Indeed, we found no differences between control and inoculated birds in melanin-based coloration of tail feathers (Hill and Brawner 1998).

Inclusion of *M. gallisepticum* in our study was accidental, and we had no *a priori* prediction for the effect of mycoplasmosis on plumage coloration other than the universal prediction that any serious illness might depress the development of a condition-dependent ornament. Experimental birds were held in captivity for at least three months, so all birds had ample time to become exposed and infected. We assumed that birds showing no clinical signs of *M. gallisepticum* infection by the end of the study were not infected. No attempt was made to physically separate birds infected with *M. gallisepticum* from birds that were not infected; attempts to do so would have been futile because

birds may be infected for several weeks before clinical signs develop.

Sixty-two percent (8 of 13) of male House Finches in 1996 and 37% (19 of 51) in 1997 showed clinical signs of infection during the study. These percentages of infected birds are very close to those of wild birds (male and female) infected (as judged by trapping) during the same time period in each year (60% in 1996, 23% in 1997; Nolan et al. 1998). The fact that some finches (both captive and wild) do not develop conjunctivitis suggests that some House Finches are resistant to infection or at least are able to suppress infection enough to prevent serious disease. The drop in the percentage of birds infected from 1996 to 1997 suggests that resistance is spreading through the House Finch population. Alternatively, the decrease in the number of clinically ill individuals in 1997 may indicate the development of a lowered virulence by the parasite. In either case, the reduced infection levels of mycoplasmosis in the 1997 trials meant that we had more birds that were MG-free, which allowed us to better assess the effects of coccidiosis on plumage color.

Our observations in 1996 indicated that male House Finches infected with *M. gallisepticum* produced plumage that is less red than that of uninfected males. In fact, many of the birds with mycoplasmosis in 1996 grew plumage that was pale yellow in coloration. Although the mechanism by which *M. gallisepticum* causes male House Finches to grow duller plumage is unknown, the disease appears to be very stressful on birds. It seems likely that for males infected with *M. gallisepticum*, energy that would have been used to produce ornamental plumage was used instead to combat infection (see Hill 2000).

Because we ended up with two parasites among our captive birds, House Finches in our experiments could have had one of four levels of parasitism: unparasitized, coccidiosis only, mycoplasmosis only, or coccidiosis and mycoplasmosis. Mycoplasmosis appears to produce a greater decrease in plumage redness than does coccidiosis. During the 1996 experiment, the mean hue score of birds with mycoplasmosis only was higher than that of birds with coccidiosis only. Furthermore, the mean hue of birds with both types of infection during the 1996 experiment was only marginally higher than that of birds with mycoplasmosis only. In other words, addition of mycoplasmosis to coccidial infections further reduced plumage redness, but mycoplasmosis alone caused nearly the same decrease in plumage redness as caused by both infections together. The strong effect of mycoplasmosis on plumage coloration could be due to the acute and very severe nature of the disease compared with the more chronic nature of coccidiosis.

Our results support the Hamilton-Zuk hypothesis (Hamilton and Zuk 1992). It has been demonstrated in experimental studies in the laboratory and the field that female House Finches prefer as mates males with the reddest and most saturated plumage (Hill 1990, 1991, 1994a; Hill et al. 1999). We have shown that males with less infection by isosporan coccidia and less severe mycoplasmal infection produce the reddest and most saturated plumage. Thus, the prediction that females should base their choice of mates on traits that indicate level of parasitism is supported. Furthermore, in the case of coccidial infections, we can be virtually certain that the decreased plumage redness was a direct effect of coccidiosis.

The importance of coccidiosis to the poultry industry has led to a great deal of research concerning the mechanism by which infection by coccidians (genus *Eimeria* rather than *Isospora* in the case of chickens) leads to decreased carotenoid pigmentation, and it has been demonstrated that coccidia disrupt carotenoid absorption (e.g. Ruff et al. 1974; Allen 1986, 1992; Tyczkowski et al. 1991). Although feather mites and pox also have been linked with decreased plumage redness in House Finches (Thompson et al. 1997), no research has been conducted to elucidate the mechanisms by which these parasites would affect plumage pigmentation. Without a mechanism that results in a direct effect on carotenoid pigmentation, we must entertain the possibility that these parasites are correlated with, but not the cause of, decreased plumage pigmentation. A male House Finch that is weakened by poor nutrition or coccidiosis (both of which can directly depress expression of plumage redness) may be less able to resist infection by mites or pox. We found that mycoplasmosis is associated with reduced expression of plumage pigmentation, but at present we do not know the mechanism by which this respiratory disease affects carotenoid pigmentation.

There has been much recent interest in whether expression of carotenoid-based plumage coloration is influenced by dietary access to carotenoid pigments (Hill 1994b, 1999; Hudon 1994; Thompson et al. 1997) or environmental stresses (Hill 1994b, Hudon 1994, Thompson et al. 1997), or whether physiological condition dictates plumage coloration independent of diet and stress (i.e. a programmed rheostasis; Bortolotti et al. 1996, Negro et al. 1998). Regarding the first two possibilities, we do not see this as a dichotomous argument. Substantial circumstantial (Hill 1993, Linville and Breitwisch 1997) and some direct evidence (Slagsvold and Lifjeld 1985; Hill 1992, 1993; Hill and Montgomerie 1994) indicates that many species of birds are limited in their access to carotenoid pigments needed to develop full expression of ornamental coloration and that the type and quantity of carotenoids ingested at least partly determines the quality of carotenoid ornamentation that is achieved. This certainly does not mean that parasitism cannot also influence expression of plumage coloration, and our results clearly show that parasites have a negative effect on the expression of carotenoid-based plumage coloration in House Finches.

Parasite infection, foraging success, and plumage coloration are likely to be tightly interlinked in species like the House Finch. Besides having a direct effect on expression of carotenoid-based plumage coloration by disrupting carotenoid absorption, transport, or metabolism, parasites may change a bird's foraging behavior and hence its intake of carotenoids and energy. Parasitized individuals will have less energy to devote to foraging and may spend relatively more time and energy seeking nutrients needed for basic maintenance versus seeking carotenoids. At the very least, parasitized birds may forage less frequently or less efficiently and ingest less food, thus ingesting fewer carotenoids (Hill and Montgomerie 1994). Conversely, good nutrition and the associated high intake of carotenoid pigments may help make individuals more resistant to parasites or better able to overcome the deleterious effects of the parasites that they do carry. It has been suggested that carotenoids themselves help boost the immune system (Lozano 1994), but it seems unlikely that even the most drably colored male House Finches are carotenoid-limited with respect to immune function (Hill 1999). Thus, females choosing brightly colored males are choosing males that must be able to cope with parasites and that also are able to ingest sufficient carotenoids for maximum expression of traits. The information provided by plumage brightness obviously would benefit females.

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