

The effect of mycoplasmosis on carotenoid plumage coloration in male house finches

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Summary

Parasites are widely assumed to cause reduced expression of ornamental plumage coloration, but few experimental studies have tested this hypothesis. We captured young male house finches *Carpodacus mexicanus* in Alabama before fall molt and randomly divided them into two groups. One group was infected with the bacterial pathogen *Mycoplasma gallicepticum* (MG) and the other group was maintained free of MG infection. All birds were maintained through molt on a diet of seeds with tangerine juice added to their water as a source of β -cryptoxanthin, the natural precursor to the primary red carotenoid pigment in house finch plumage. All males grew drab

plumage, but males with MG infection grew feathers that were significantly less red (more yellow), less saturated, and less bright than males that were not infected. MG targets upper respiratory and ocular tissue. Our observations show that a pathogen that does not directly disrupt carotenoid absorption or transportation can still have a significant effect on carotenoid utilization.

Key words: sexual selection, plumage coloration, carotenoid, parasite, house finch, *Carpodacus mexicanus*, *Mycoplasma gallicepticum*.

Introduction

It is widely assumed that parasites affect expression of ornamental coloration in animals and that color displays serve as honest indicators of parasite resistance (Hamilton and Zuk, 1982). This assumption is supported primarily by correlations between ornament expression and parasite load measured in wild animals (for a review, see Hamilton and Poulin, 1997). In only relatively few studies have the effects of parasites on ornament expression been studied through carefully controlled infection experiments. Zuk et al. (1990) conducted infection experiments on red-jungle fowl *Gallus gallus* with intestinal roundworms *Ascaridia galli*, and showed that roundworm infection reduced comb size in males. Moreover, in a field experiment with barn swallows *Hirundo rustica*, Møller (1990) showed that males infected with blood-sucking mites grew shorter tails than males that were not infected. These experimental studies show that parasites can affect expression of morphological traits. The effects of parasites on animal coloration, and especially the brilliant plumage coloration of birds, is of special interest because it was plumage coloration that was the focus of the original statement of the Hamilton–Zuk hypothesis (Hamilton and Zuk, 1982) that ornamental traits evolve as indicators of parasite resistance.

Carotenoid pigmentation is one of the most widespread mechanisms for ornamental coloration, particularly in fish and birds (Goodwin, 1984), and carotenoid pigmentation has become a text-book example of a condition-dependent display trait (e.g. Gill, 1995; Alcock, 2001). Carotenoids are a class of

molecules that cannot be synthesized by vertebrates – they must be ingested to be used as integumentary pigments (Völker, 1934, 1938). Expression of carotenoid-based ornamental coloration is thus partly a function of the type and quantity of pigments that are ingested (Hill, 2002). Once carotenoids are ingested, however, they still have to be properly utilized to create ornamental coloration, and parasites can disrupt this utilization process. Previous experimental studies have shown that monogenean parasites and the protozoan parasite, *Ichthyophthirius multifiliis*, can affect expression of carotenoid pigmentation in guppies *Poecilia reticulata* and three-spined stickleback *Gasterosteus aculeatus*, respectively (Milinski and Bakker, 1990; Houde and Torio, 1992). Field correlational studies of yellowhammers *Emberiza citrinella* (Sundberg, 1995) and greenfinches *Carduelis chloris* (Merila et al., 1999) suggest that parasites can affect expression of carotenoid-based plumage coloration in birds. Controlled aviary experiments with American goldfinches *Carduelis tristis* also showed that coccidiosis (McGraw and Hill, 2000) and mycoplasmosis (Navara and Hill, 2003) can affect expression of ornamental carotenoid coloration.

In this study we tested the effects of the bacterium *Mycoplasma gallicepticum* (MG) on expression of plumage coloration in the house finch *Carpodacus mexicanus*, a species in which males have carotenoid-based ornamental coloration that varies from pale yellow to bright red (Hill, 1993). Several

studies have been conducted on the effects of parasites on expression of plumage coloration in the house finch. Thompson et al. (1997) found that males that were infected with avian pox prior to molt grew feathers that were less brightly pigmented compared to males that did not have pox infection prior to molt. They found a similar relationship between feather mites and coloration (Thompson et al., 1997). Brawner et al. (2000) conducted an experiment to test the effect of *Isospora* coccidia on plumage coloration. They found that males that they infected with *Isospora* during molt grew drabber plumage than males that were maintained free of coccidial infection. In this experiment, some birds contracted mycoplasmal conjunctivitis and birds with MG grew drabber plumage than birds that were free of MG.

Although these studies represent a substantial body of work on the effects of parasites on carotenoid-based plumage coloration, and the house finch has been the focus of more research than any other passerine bird, fundamental questions remain regarding the effects of parasites on carotenoid-based plumage coloration in the house finch. First, the only carefully controlled experiment looking at the effects of parasites on plumage coloration was conducted with isosporan coccidia. Coccidia are parasites of the gastrointestinal tract. They are known to directly inhibit carotenoid absorption and the production of carotenoid carrier proteins (Allen, 1987a,b). Thus, coccidiosis is a disease that is expected to have direct effects on expression of carotenoid-based plumage coloration. The effects on plumage coloration of diseases that are more systemic and that are not known to directly inhibit carotenoid uptake have not been tested experimentally. In the Brawner et al. (2000) study, MG broke in cages of birds – individuals were not assigned to treatment groups. Whether or not individual birds became infected was likely to have been related to their ability to resist the pathogen. Thus, the effect of parasites is confounded by the overall health and condition of individual birds. Finally, in previous aviary studies looking at the effects of parasites on plumage coloration in house finches, males were maintained on diets supplemented with the red carotenoid canthaxanthin (Brawner et al., 2000). Canthaxanthin is used by male house finches directly as a plumage pigment without being modified (Inouye et al., 2001; Hill, 2002). The dominant red pigment in the plumage of wild male house finches, however, is 3-hydroxy-echinenone, which is the metabolic derivative of the dietary pigment β -cryptoxanthin (Inouye et al., 2001). By feeding males a red pigment and bypassing metabolic pathways, previous feeding experiments may have underestimated the effects of parasitism on plumage coloration.

In the present study we tested the effects of *Mycoplasma gallicepticum* on expression of plumage coloration in male house finches. Two groups of males were maintained on a diet supplemented with β -cryptoxanthin. One group was experimentally infected with MG while the other group was maintained free of MG through the molt period. This study was designed as an experimental test of the effects of a systemic

infection on expression of carotenoid pigmentation when birds are utilizing a natural dietary precursor for plumage pigments.

Materials and methods

We captured hatch year house finches *Carpodacus mexicanus* Müller in Auburn, Alabama, USA from early July through August. Upon capture, we took 100 μ l of blood from each bird. Blood was spun to separate plasma and red blood cells, which were stored in TNE buffer at -80°C . Plasma was stored at 4°C for serological analysis (see below). In juvenile plumage, male and female house finches cannot be distinguished morphologically, so we used a molecular-sexing technique to determine the sex of the birds that we captured. Briefly, the DNA was extracted from stored red blood cells using a standard phenol/chloroform technique (Quinn and White, 1987; Westneat, 1993). Extracted DNA was resuspended in TE buffer and stored at -20°C . We identified the sex of the hatch year birds using P2 and P8 microsatellite primers and the PCR protocol outlined by Griffiths et al. (1998). These primers amplify introns on the *CHDI-W* and *CHDI-Z* genes. Two bands are present in females, which are the heterogametic sex, whereas males as the homogametic sex have a single band (Griffiths et al., 1998). PCR products were separated on a 1.5% agarose gel laced with ethidium bromide by electrophoresis at 150 V for 2 h.

Males were housed in small cages with 2 birds per cage for the duration of the experiment. Birds within a cage received the same experimental treatment – either infected or not infected (see below). They were held near windows so they experienced a natural light cycle. All birds had *ad libitum* access to a canary pellet diet (canary maintenance, Avi-Sci Inc., St Johns, Michigan, USA). Through the molt period, we added one part tangerine juice (100% pure juice, not from concentrate, never frozen) to one part drinking water for all birds. Tangerine juice tended to spoil at room temperature so tangerine juice/water was changed every 24 h.

To ensure that none of the birds in our experiments had been previously exposed to MG, we tested the serum of each bird for antibodies to MG using a serum plate agglutination assay as described in Roberts et al. (2001). We also tested birds for the presence of MG by polymerase chain reaction (PCR; Roberts et al., 2001). We collected samples for analysis by PCR by swabbing the choanal cleft using a micro-tip swab (Becton Dickinson and Co. Maryland, USA). Any birds that were found to have antibodies to MG or that were PCR-positive were excluded from the study. Coccidiosis is another widespread disease of house finches that is known to affect expression of carotenoid coloration (Brawner et al., 2000). To be certain that coccidiosis did not confound the effects of mycoplasmosis in this experiment, we added sulfadimethoxine to the water of all birds to ensure that they remained free of coccidiosis (Brawner et al., 2000).

We cultured MG from symptomatic wild house finches caught in Auburn, Alabama. We infected the birds in the MG treatment group by dropping 10 μ l of SP4 medium containing 1×10^6 color-

changing units ml^{-1} into each eye for a total dose of 2×10^4 color-changing units. This dose of MG has been effective in previous studies in inducing a modest infection among captive house finches (Roberts et al., 2001). Birds in the uninfected treatment group were sham infected with the same amount of sterile SP4. We monitored the birds daily for onset of disease. Disease was measured for each eye on a five-point scale, where 0=normal eye and 4=blindness caused by swelling (Roberts et al., 2001). We captured all birds three weeks post-inoculation to collect blood for serology and swabs for MG detection by PCR.

Following molt, we measured plumage coloration of all males using a Colortron reflectance spectrophotometer (Hill, 1998). Male house finches display carotenoid-based plumage coloration on their crown, breast and rump, and a technician with no knowledge of the experiment scored plumage color by taking three measurements in each of these areas. We averaged these measurements to obtain an overall hue, saturation and brightness for each male. We photographed the breast patch of each male along with a size standard and used Sigma Scan 5.0 to measure breast patch size. We calibrated each picture using the size standard and then traced the breast patch three times and used the average size in all analyses.

All infection protocols carried out in this study were approved by the Institutional Animal Care and Use Committee (IACUC) at Auburn University. We used the smallest sample of birds that would give us reasonable power to detect differences among groups.

Results

Birds in the uninfected group remained free of MG throughout the study. No birds in the uninfected group showed symptoms of mycoplasmosis nor did any birds show a positive antibody response to MG. All PCR tests of birds in the uninfected group were negative.

All birds that were inoculated with MG developed conjunctivitis in both eyes within 10 days of inoculation. All birds in this group developed antibodies to MG and all tested positive for MG by PCR. For most birds the infection lasted throughout the 8-week molt period, with the most severe clinical symptoms occurring 2–6 weeks after infection.

After molt on the pellet diet supplemented with tangerine juice (as a source of β -cryptoxanthin; Hill, 2000), all males grew pale orange plumage, much drabber than the average wild male in the Auburn, Alabama population. There was a significant effect of MG infection on ornamental plumage coloration. Males that were infected with MG during molt grew breast feathers that were more yellow/less red ($Z = -1.90$, $N = 15, 16$, $P = 0.03$), less saturated ($Z = -1.86$, $N = 15, 16$, $P = 0.03$), and less bright ($Z = -1.78$, $N = 15, 16$, $P = 0.04$) than males that had no MG infection (Mann–Whitney U Tests) (Fig. 1). There was no significant effect of the cage in which males held on any component of plumage coloration (hue: F -ratio=1.62, $P > 0.17$; saturation: F -ratio=1.84, $P > 0.12$; brightness: F -ratio=1.33, $P = 0.29$).

Patch size was measured on males several months after molt

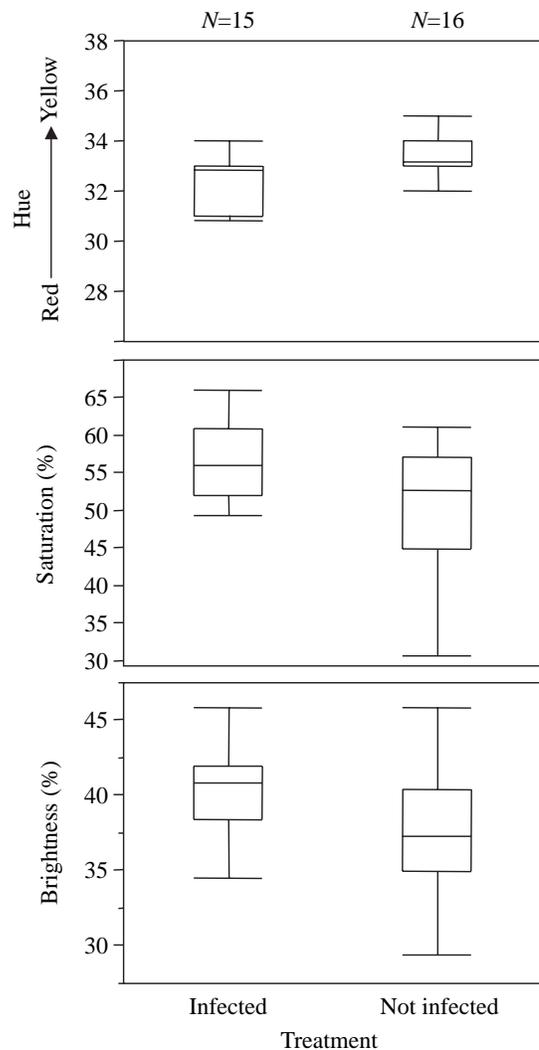


Fig. 1. The effects of *Mycoplasma gallicepticum* (MG) infection on expression of carotenoid plumage coloration in male house finches. Plotted as horizontal lines are the median and 10th, 25th, 75th and 90th percentiles of the hue, saturation and brightness. Comparisons were made with the mean color of the crown, breast and rump feathers for each male following molt within experimental treatment group. Infected males all had conjunctivitis during the molt period. Uninfected males tested negative for MG both in antibody tests and PCR tests at capture and were held in a quarantine facility away from sources of MG during molt. All birds were fed a low-carotenoid pellet diet and water supplemented with tangerine juice as a source of carotenoid pigments.

and by that time several males had died or been used in other experiments. Therefore we had only 12 infected and 13 control males for patch size comparison. We found no significant difference in the patch sizes of male that were infected with MG during molt and the patch sizes of males that were not infected ($Z = -0.65$, $P = 0.51$).

Discussion

Understanding the effects of parasites on production of

ornamental traits is central to a general understanding of the signal function of these displays. Signaling parasite resistance has been proposed to be a key function of color displays and to have been the driving force in the evolution of such traits (Hamilton and Zuk, 1982). This theory relies crucially on parasitic infection reducing the magnitude of display traits. Despite the importance of the hypothesis that parasites reduce expression of display traits, few experimental studies have been conducted to test this idea.

Previous research had shown that in controlled infection experiments, coccidiosis caused male house finches to grow less red and less saturated plumage coloration (Brawner et al., 2000). In these infection experiments that controlled the exposure of males to coccidiosis, some males contracted mycoplasmal conjunctivitis, and it was found that males with mycoplasmal conjunctivitis grew less red, less saturated, and less bright plumage than males that were not infected (Brawner et al., 2000). Because males in the Brawner et al. (2000) study were not infected with MG by researchers, but rather either contracted or resisted contracting the disease, there is the chance that male health and condition affected both plumage coloration and disease state.

In the present study we eliminated the uncertainty of previous studies and show definitively that mycoplasmal conjunctivitis during molt depressed expression of ornamental plumage coloration in male house finches. In our experiment, we randomly assigned males to treatment groups eliminating any confounding effects of condition or health. We also fed males the precursor to the red pigment that is the most abundant red pigment in the plumage of wild house finches. By feeding the metabolic precursors to red feather pigments, we forced birds to include more steps in the utilization of pigments. Interestingly, even though we forced birds to metabolically modify dietary pigments to produce red ornamental coloration, and thus added at least one step to the process of carotenoid utilization, we saw no greater effect of MG on plumage coloration in this study, compared to the previous study in which males were fed red feather pigments directly (Brawner et al., 2000).

We found a significant negative effect of mycoplasmosis on plumage coloration, but no significant effect of mycoplasmosis on the size of carotenoid breast patches. This observation is consistent with observations from feeding experiments in which access to carotenoid pigments had a large effect on plumage coloration but a small effect on patch size (Hill, 1992, 1993). This finding is also consistent with the idea that the quality of ornament pigmentation (coloration) and the area of the body with pigment (patch size) are under distinct developmental control, with largely independent responses to environmental challenges and different signaling function (Badyaev et al., 2001).

As a source of β -cryptoxanthin, we fed males in this experiment tangerine juice (Hill, 2000). β -cryptoxanthin is the predominant carotenoid in tangerine juice (Mangels et al., 1993), but the amount of β -cryptoxanthin ingested by male house finches in this study was probably still small

compared to the amount of the pigment that is likely to be ingested by wild finches feeding on fruits. Males in both treatment groups appeared to have fewer pigments available than they needed for maximum expression of ornamental coloration, and all males were drab at the end of the feeding experiment. Experimentally infected males were simply drabber on average than uninfected males. We assume that we may have seen larger differences in plumage coloration between control and infected groups if males had been fed larger doses of β -cryptoxanthin, but it is also conceivable that access to more β -cryptoxanthin may have masked the effects of mycoplasmosis.

Previous experimental studies of parasites and plumage coloration in house finches have focused on coccidiosis. In one sense, these studies are particularly valuable because they focus on a parasite for which a mechanism for direct inhibition of carotenoid utilization is known (Brawner et al., 2000). On the other hand, studies of coccidia leave open the question of the effects of parasites that do not directly inhibit carotenoid uptake or transport, but that have more general systemic effects on the bird and would have indirect effects on pigment utilization. MG is an upper respiratory disease (Jordan, 1996). It does not infect gastro-intestinal tissues and thus it is unlikely to directly affect carotenoid absorption. Nevertheless, we found that MG depressed expression of ornamental coloration. The present study, coupled with previous experiments on the effects of disease on house finches, shows that both parasites that specifically target gastro-intestinal tissues and directly disrupt carotenoid utilization as well as parasites that infect tissues outside the gastro-intestinal track can have a significant effect on how carotenoid pigments are utilized. The relative importance of these two types of parasites on expression of plumage coloration among males in wild populations remains to be determined.

One intriguing possibility is that, in birds infected with MG, carotenoids that could have been used for ornamental display were instead diverted to bolster the immune system against the infection (Lozano, 1994; Møller et al., 2000). We cannot assess this hypothesis with the data at hand, but it has yet to be shown for any species that there is a trade-off between use of carotenoids for ornamental plumage coloration and use of carotenoids for immune defense (Hill, 1999). A recent study on American goldfinches failed to find such a trade-off in a carefully controlled infection experiment (Navara and Hill 2003). A simpler explanation is that the house finches in this study that were infected with MG diverted energy away from pigment utilization to immune defense and this diversion of energy caused the loss of plumage coloration in infected males.

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