Yolk Antioxidants Vary with Male Attractiveness and Female Condition in the House Finch (*Carpodacus mexicanus*)

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ABSTRACT

The manipulation of egg content is one of the few ways by which female birds can alter offspring quality before hatch. Lipid-soluble vitamins and carotenoids are potent antioxidants. Female birds deposit these antioxidants into eggs in variable amounts according to environmental and social conditions, and the quantities deposited into eggs can have effects on offspring health and immunological condition. Allocation theory posits that females will alter the distribution of resources according to mate quality, sometimes allocating resources according to the differential allocation hypothesis (DAH), investing more in offspring sired by better-quality males, and other times allocating resources according to a compensatory strategy, enhancing the quality of offspring sired by lower-quality males. It is unknown, however, whether antioxidants are deposited into eggs according to the DAH or a compensatory strategy. We examined deposition patterns of yolk antioxidants (including vitamin E and three carotenoids) in relation to laying order, mate attractiveness, female condition, and yolk androgen content in the house finch (*Carpodacus mexicanus*). Female house finches deposited significantly more total antioxidants into eggs sired by less attractive males. Additionally, yolk antioxidant content was significantly positively correlated with female condition, which suggests a cost associated with the deposition of antioxidants into eggs. Finally, concentrations of antioxidants in egg yolks were positively correlated with total yolk androgen content. We suggest that yolk antioxidants are deposited according to a compensatory deposition strategy, enabling females to improve the quality of young produced with less attractive males. Additionally, yolk antioxidants may act to counter some of the detrimental effects associated with high levels of yolk androgens in eggs and, thus, may exert a complementary effect to yolk androgens.

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Introduction

For female birds, an important aspect of parental investment is the resources allocated to eggs. The resources available to any female for reproduction and self-maintenance will be finite, and she will be inevitably faced with decisions regarding how much resource to invest in each egg in each clutch that she lays. Burley (1988) theorized that females would allocate resources in such a way that would maximize the number and quality of offspring. In this discussion, she proposed the differential allocation hypothesis (DAH), which states that females should invest more in offspring sired by better-quality males, trading off between current and future reproductive attempts and maximizing overall reproductive success. Support for the DAH comes from observations of clutch size (Petrie and Williams 1993), parental feeding rates (de Lope and Møller 1993), and egg size (Cunningham and Russell 2000) in which females invested more in offspring sired by more attractive males. In other studies, however, the reverse pattern was found—females contributed more to offspring sired by lower-quality males (Michl et al. 2005; Navara et al. 2006*a*), contradicting the predictions of the DAH. Navara et al. (2006*a*) suggested that higher investment in the offspring of lower-quality males may represent a compensatory strategy, whereby females make the most of the current reproductive attempt by contributing more to offspring sired by lower-quality males.

Allocation hypotheses have recently been expanded to explain patterns of androgen content in avian eggs. Specifically, female zebra finches and canaries deposit androgens according to patterns consistent with the DAH (Gil et al. 1999; Tanvez et al. 2004), where androgen content of eggs is higher with the perception of a good-quality mate. The DAH, however, requires that the investment be a resource that is costly to the female and/or her offspring and beneficial in terms of fitness (Sheldon 2000), and previous work suggests that androgens are not resources but are instead physiological mediators that have a variety of effects on offspring (Schwabl 1996; Groothuis et al.

2005*a*, 2005*b*; Navara et al. 2005, 2006*b*). Perhaps androgens are not the best component of eggs on which to test allocation theories. Indeed, the DAH does not always explain patterns of yolk androgen deposition, as female collared flycatchers deposit more androgens into eggs sired by younger, lower-quality males (Michl et al. 2005), a pattern that is inconsistent with the DAH. Thus, it would be interesting to examine other components of egg yolks that are variably deposited and are true resources. Such an analysis should provide a better test of whether females strategically deposit resources into eggs according to the DAH or according to a compensatory strategy.

Antioxidants, including a range of lipid-soluble vitamins (i.e., vitamins A and E) and carotenoids, are potentially important resources deposited by female birds into eggs. Carotenoids are biologically active pigments that cannot be synthesized by birds and must instead be ingested. Carotenoids deposited into the yolk are transferred into the embryonic liver and protect against potentially harmful oxidative effects that occur as a result of developmental processes (Surai and Speake 1998; Surai et al. 1999). Supplementation of female hens with high levels of dietary carotenoids resulted in a significant increase in yolk carotenoid levels of eggs, a reduction in the susceptibility of yolk and chick samples to in vitro peroxidation (Surai and Speake 1998), and enhanced immune function of hatchlings (Haq et al. 1996). Similarly, vitamins A and E also have antioxidant capabilities (McCay 1985; Keys and Zimmerman 1999) and are shuttled from the avian yolk to the embryonic liver during the incubation period (Gaal et al. 1995).

Recent work has documented distinct within- and among-clutch variation of antioxidant concentrations in avian eggs. Royle et al. (1999) showed that lesser black-backed gull eggs that were laid earlier in the laying sequence had greater quantities of vitamin E and carotenoids than those laid later in the sequence. Female barn swallows deposited significantly higher concentrations of the carotenoid lutein into eggs sired by males with experimentally shortened tails. Because tail length in barn swallows is an indicator of both attractiveness and parasite susceptibility, females may be utilizing yolk antioxidants as a means of compensating for immunological deficiencies of offspring sired by lower-quality males (Saino et al. 2002). Thus, female birds appear to deposit antioxidants into eggs based on environmental and social conditions. Patterns of vitamins and carotenoids in avian eggs are, however, still understudied.

We examined patterns of yolk antioxidants (including vitamin E, vitamin A, and three carotenoids) in relation to laying sequence, male attractiveness, female condition, and yolk androgen content in wild house finches (*Carpodacus mexicanus*) to determine whether females deposit these resources according to the DAH or according to a compensatory strategy. Female house finches often begin incubating before the last egg is laid, resulting in offspring that hatch asynchronously (Badyaev et al. 2003). As a result, house finch nestlings hatching from eggs in the fifth clutch position have been shown to be significantly smaller and less likely to survive (Dervan 2001), and the adjustment of laying order and thus of differential maternal allocation in relation to laying order in this species may have a strong adaptive value (Badyaev et al. 2002*a*, 2002*b*). Further, up to 50% of the variation in offspring phenotype is accounted for by maternal effects in this species (Badyaev 2005). Male house finches express carotenoid-based plumage coloration ranging from bright red to drab yellow, which can only be produced using carotenoids ingested through dietary means as house finches cannot produce carotenoids de novo (Goodwin 1984). Females in our population show a mating preference for males with redder, more saturated plumage (Hill 1990, 1991), and redder males provision at a higher rate, providing direct benefits to females and their offspring (Hill 1991). Male color has also been positively correlated with number of offspring fledged (McGraw et al. 2001) and overwinter survival (Hill 1991), and it has been shown that brighter red males pair with older, more attractive females (Hill 1993). Importantly, female house finches deposit more androgens into eggs sired by less attractive males and into eggs laid later in the clutch, potentially following a compensatory strategy of androgen deposition (Navara et al. 2006*a*).

We predicted that, if females deposit antioxidants according to a compensatory strategy similar to that seen for androgen deposition, eggs laid later in the sequence and those sired by less attractive males would receive more antioxidants. The reverse would be true if female house finches deposit antioxidants according to the DAH. Additionally, we predicted that, if deposition strategies for androgens and antioxidants were similar, yolk antioxidant concentrations would be positively correlated with yolk androgen concentrations.

Material and Methods

Male Color and Female Condition

We monitored a nesting population of house finches in Lee County, Alabama. The field site contained approximately 150 nest boxes within a 25-mi^2 area, most of which were occupied by nesting house finches. We examined patterns of allocation of yolk androgens at each nest in relation to the female condition and the color of the attending male. Nesting male and female house finches were captured in baited traps. Males have carotenoid coloration on the head, breast, and rump. Thus, three color measurements were taken from each of those regions and averaged to produce a mean set of color measurements for each body area. Color measurements were made using a Colortron reflectance spectrophotometer (Hill 1998), which calculates three separate aspects of color: hue, saturation, and brightness. Measurements of separate body parts were averaged to provide an overall average measurement for each color variable. The average values for the three male color variables were analyzed using a principal components analysis (PCA). The variation in plumage coloration was explained by

three principal components. The first component (PC1) explained 46% of the variation with an eigenvalue of 1.39 and had a strong positive loading from hue (0.83) and brightness (0.60) and a strong negative loading from saturation (−0.70). Thus we used this component in our assessment of male "attractiveness." The Colortron measures hue as position on a color wheel, where scarlet is arbitrarily assigned a value of 0 and yellow is assigned a value around 20. So male house finches with higher saturation but lower hue scores are more ornamented. For our comparisons, we divided males into two groups—above the median and below the median. There was a natural break in the distribution of the principal component values at the median value, which occurred at 0. Males with a PC1 below 0 will hereafter be referred to as "more attractive" males, and males with a PC1 above 0, which were less ornamented, will hereafter be referred to as "less attractive."

Female tarsus length was measured using manual dial calipers (accuracy = 0.01 mm), and mass was measured using a digital scale (accuracy = 0.05 g). The residuals of mass : tarsus length were calculated as a measure of condition in these females. These measurements incorporate both skeletal size and mass of the animal and generate an excellent estimation of individual avian condition, and the residuals of these measurements provide a clean method of separating the effects of condition from the effects of body size (Reist 1985).

Egg Collection

After the appearance of the first egg, we used visual assessments to determine the date of incubation onset. Previous experiments using nest temperature measurements showed that visual assessments consistently and accurately estimated the day of incubation onset (Badyaev et al. 2003). All eggs were retrieved after 36 h of incubation, before the development of embryonic gonadal tissue (K. Navara, unpublished data), and frozen at −20°C. In cases where incubation began before the last egg was laid, each egg within the nest was collected 36 h after its incubation onset, resulting in a similar incubation time for all eggs. Because previous studies have shown that concentrations of yolk androgens decrease after the onset of incubation (Elf and Fivizzani 2002; Rutstein et al. 2005), we were careful to collect all eggs after the same period of incubation, thus eliminating variation resulting from the period of incubation itself. We were able to obtain full data, including yolk androgen concentrations, male color, and female condition, on 15 nests.

Yolk Androgen Analyses

The albumin, yolk, and the small embryo were separated by thawing. Embryos in collected eggs were of comparable sizes at this stage of development. Yolks were homogenized, and 20–40 mg of the homogenate was diluted in 1 mL of water for the analysis. Yolk testosterone (T), androstenedione (A4), and dihydrotestosterone (DHT) were separated by celite column chromatography according to methods described by Schwabl (1993). T and A_4 were quantified using a standard competitive binding radioimmunoassay, using a specific antibody (Esoterix, Calabasas Hills, CA) according to methods outlined by Mendonça et al. (1996). DHT was quantified using a commercial I^{125}-labeled radioimmunoassay kit from Diagnostics Systems Laboratories (Webster, TX). Interassay variation was 20% for A_4, 3% for T, and 14% for DHT, and intra-assay variation was 6% for A_4 and 4% for T. Assay lower detection limits were 20 pg mL^{-1} for A_4 and T and 25 pg mL^{-1} for DHT. Because all androgen concentrations were highly correlated with one another ($P < 0.0001$ in all cases) and, in most cases, all androgens showed similar patterns when analyzed separately, we used the sum of all three androgens in our analyses.

Yolk Carotenoid and Vitamin Analyses

Yolk carotenoids, including lutein, β-cryptoxanthin, and β-carotene, and four variants of vitamin E (α-, β-, δ-, and γ-tocopherol) were measured using high-performance liquid chromatography (HPLC). Weighed amounts of yolk (0.05–0.10 g) were vortexed and homogenized in 75 : 25 methanol containing pyrogallol (2% w/v)-water in a volume equaling 10 times the yolk sample mass. Samples were incubated in 10% potassium hydroxide at 70°C for 1 h, vortexing every 15 min. Triple extraction with hexane (2 mL) was followed by washing of the organic phase with water (0.5 mL). Samples were centrifuged at 1,700 *g* for 5 min between extractions, and the combined organic phase was evaporated to dryness under vacuum at 40°C and reconstituted in 300 μL ethyl ether and 900 μL HPLC mobile phase (methanol-acetonitrile-tetrahydrofuran, 50 : 45 : 5 v/v/v).

Carotenoids and vitamins were quantified by injecting 50 μL of yolk extract into an HPLC System (Shimadzu, Pleasanton, CA) fitted with a NovaPak C18 column, 150 × 3.9 mm (Waters Corporation, Milford, MA). The initial diversity and prevalence of carotenoid and vitamin compounds were assessed by Craft Technologies (Wilson, NC). Subsequent analyses focused on the identified compounds. Analytes were eluted isocratically at a constant flow rate of 1 mL min^{-1} for 22 min using the aforementioned mobile phase. Carotenoids, retinoids, and tocopherols were detected using a Shimadzu SPD-M10AVP photodiode array detector, and peak areas were integrated at 450, 325, and 294 nm, respectively. Peaks were identified and quantified (μg g^{-1}) using retention times and calibration curves of standards (Sigma, St. Louis, MO; Supelco, Bellenote, PA; Indofine Chemical, Hillsborough, NJ; CaroteNature, Lupsingen, Switzerland).

For all analyses involving individual antioxidants, we used the sum of the four variants of vitamin E (α-, β-, δ-, and γ-tocopherol) as total vitamin E concentration and the sum of all carotenoids (lutein, β-cryptoxanthin, and β-carotene) as

total carotenoid concentration. Additionally, we ran similar analyses using the sum of all antioxidants (hereafter referred to as total antioxidants). Total vitamin E, carotenoids, and antioxidants were used in these analyses in order to decrease the number of statistical tests performed and also because an assessment of total relevant antioxidant function would include all of these protective molecules in combination. Thus the potentially additive effects of all antioxidants measured makes it important to examine them together.

Statistical Analyses

The relationship between average total and individual yolk antioxidants in eggs sired by attractive and unattractive males was tested using an ANOVA. All nests included in these analyses contained eggs from individual pairs of birds that were not repeated in the analyses. Two of the pairs included in these analyses had more than one clutch during the season, so we selected the clutch with the most eggs for the analyses in order to maximize sample sizes for the analyses of laying order effects. Results were similar when using a standard method of including all first clutches in the analyses as well. Average total and individual yolk antioxidants for each clutch were examined in relation to female condition using the residuals of the regression of female mass : tarsus length, and comparisons were made using a simple regression. Variation in total and individual yolk androgen content using clutch averages in relation to the month in which the eggs were laid was analyzed using an ANOVA. Clutch averages of total and individual yolk androgens were used in these analyses to avoid using the same females in duplicate analyses. Additionally, patterns of total and individual yolk antioxidants were examined in relation to clutch position using an ANOVA. Finally, patterns of total and individual yolk antioxidants were analyzed in relation to total yolk androgens (defined as the sum of yolk A_4, T, and DHT) using simple regressions.

Results

House finch eggs contain variable amounts of androgens, carotenoids, and vitamins. Testosterone was the most abundant androgen in house finch yolk (mean = 7.0 pg mg^{-1}) followed by A_4 (mean = 2.0 pg mg^{-1}) and DHT (mean = 1.12 pg mg^{-1}). The most abundant carotenoid we measured in house finch yolk was lutein (mean = 8.0 $\mu g\ g^{-1}$ yolk) while β-cryptoxanthin and β-carotene were present in low levels (means = 0.78 and 0.01 $\mu g\ g^{-1}$ yolk, respectively). Vitamin E was the most abundant vitamin in house finch yolk (mean total vitamin E = 169.1 $\mu g\ g^{-1}$ yolk), and the α-tocopherol form was the most prevalent form of vitamin E (means: α-tocopherol = 145.97 $\mu g\ g^{-1}$, β-tocopherol = 10.35 $\mu g\ g^{-1}$, γ-tocopherol = 6.67 $\mu g\ g^{-1}$, δ-tocopherol = 3.02 $\mu g\ g^{-1}$).

House finch females deposited significantly more total antioxidants into clutches of eggs sired by less attractive males ($F_{1,16} = 9.28$, $P < 0.01$; Fig. 1). When these results were broken down into individual antioxidants, vitamin E was significantly higher in eggs laid by females mated to less attractive males ($F_{1,16} = 7.46$, $P = 0.015$). In fact, eggs sired by unattractive males had approximately 2.5 times the vitamin E levels of eggs

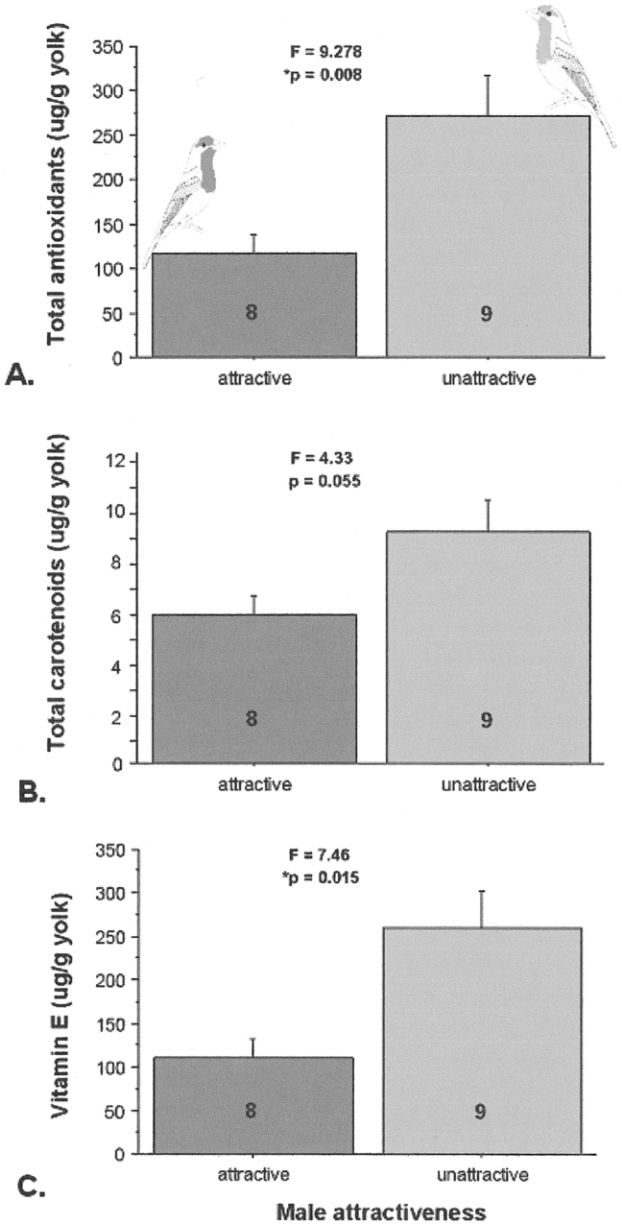

Figure 1. Yolk antioxidant concentrations (including all measured carotenoids and vitamins), expressed as clutch averages (error bars show SEs) in eggs sired by less attractive male house finches versus eggs sired by more attractive male house finches. Numbers located inside the bars indicate the number of males analyzed in each attractiveness group.

sired by attractive males. Additionally, a similar trend approached significance for total carotenoids ($F_{1,16} = 4.33$, $P = 0.055$).

Total antioxidant concentration in yolk was also significantly positively correlated to our measure of female condition ($r^2 = 0.482$, $P = 0.02$; Fig. 2). When analyzing each antioxidant separately, however, both total carotenoids and vitamin E showed similar but nonsignificant trends. Female condition was not significantly associated with the attractiveness of her mate ($F_{1,13} = 0.51$, $P = 0.50$).

Total antioxidant concentration did not differ across the laying order ($F_{4,80} = 0.922$, $P = 0.46$; Fig. 3), and these results were similar when analyzed using individual antioxidants. Total antioxidant concentration was significantly positively correlated with total androgen concentrations in the yolks of house finch eggs ($r^2 = 0.586$, $P < 0.001$), and this significant correlation also existed for antioxidants when analyzed separately (total carotenoids, $P < 0.001$; vitamin E, $P < 0.001$).

Discussion

The patterns of antioxidant allocation to eggs that we observed in our population of house finches are consistent with a compensatory strategy of resource allocation but not with the predictions of the DAH. Female house finches deposited significantly more antioxidants into eggs sired by unattractive males, and females in better condition deposited more antioxidants into their eggs. Additionally, total antioxidant concentration of egg yolks was significantly positively correlated with total yolk androgen concentration of eggs. These patterns suggest that yolk antioxidants are used to mitigate the detrimental conditions experienced by offspring sired by less attractive house finch males, which have been shown to provide less food to offspring (Hill 1991, 2002).

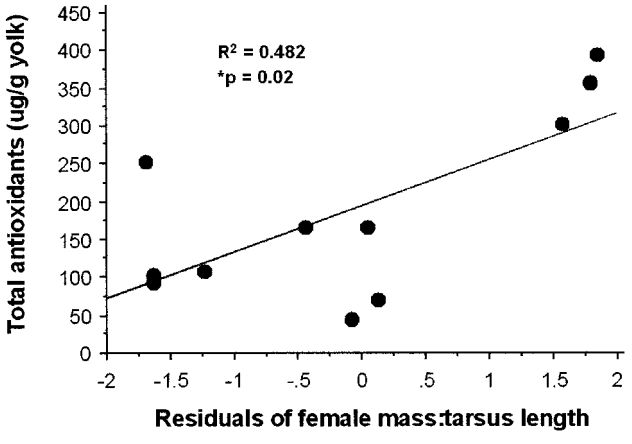

Figure 2. Total yolk antioxidant concentrations (including all measured carotenoids and vitamins), expressed as clutch averages, in relation to condition index of the resident female.

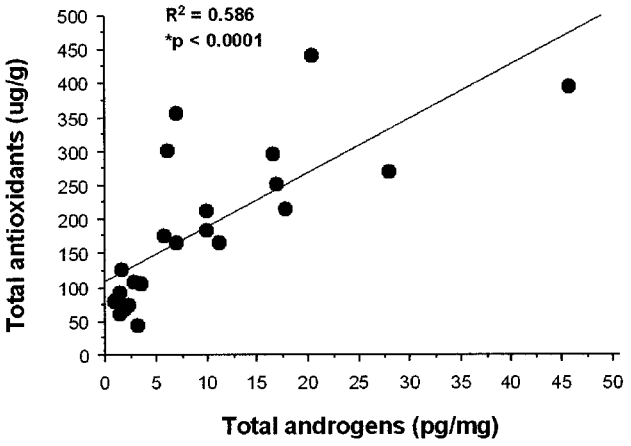

Figure 3. Total yolk antioxidant concentrations (including all measured carotenoids and vitamins) in relation to total yolk androgens (including testosterone, androstenedione, and dihydrotestosterone). All values are expressed as clutch averages.

If female house finches use antioxidants as a means to compensate for detrimental effects associated with hatching asynchrony, then we would expect that eggs laid later in the clutch would contain higher concentrations of antioxidants. While testosterone concentrations increased with laying order in house finch eggs (Navara et al. 2006*a*), yolk antioxidant concentrations were similar for all laying positions. In the current experiment, sample sizes were not as large as those in which testosterone concentrations were analyzed (Navara et al. 2006*a*). Perhaps our sample sizes were not large enough to detect the subtle within-clutch trends that may be exhibited by antioxidant levels in house finch yolks. Or, it is possible that, as a result of the 36-h time period between egg laying and collection, subtle variations in antioxidant concentrations among eggs within clutches were eliminated because of antioxidant metabolism. All eggs were collected after similar periods of incubation, however, which should eliminate variation due to incubation time. Alternatively, the differences we see in the patterns of hormones and antioxidants in relation to laying position may have resulted from the manner in which these two potent substances benefit offspring. Androgens deposited into the yolk have been shown to increase growth (Schwabl 1996; Groothuis et al. 2005*b*; Navara et al. 2005) and begging rates of offspring (Schwabl et al. 1997), which could compensate for the potentially detrimental effects associated with hatching from an egg in a later clutch position. Perhaps the effects of hatching asynchrony on oxidative stress and immunological condition are not severe enough to cause selection for a within-clutch gradient of yolk antioxidant deposition.

The relationships between yolk antioxidants and both male color and female condition argue against the arbitrary deposition of antioxidants. Dietary antioxidants are important to the maintenance of an oxidant : antioxidant balance within the

body, especially under conditions of high oxidative stress (Chew and Park 2004). Reactive oxidative products, when left uncontrolled, can cause extensive physiological damage, including neurodegenerative disease (Sun and Chen 1998), mitochondrial dysfunction (Richter and Kass 1991), and apoptosis of normal cells (Buttke and Sandstrom 1994; Sun and Chen 1998), including cells of the immune system (Bendich 1989). In fact, normal cellular activity of lymphocytes and macrophages includes the generation of reactive oxidative products, increasing the likelihood of free-radical exposure for cells of the immune system. Thus, the activity of the reactive oxidative products produced by immune cells must be carefully controlled by both endogenously produced and dietary antioxidants (Chew and Park 2004) to avoid a detrimental immunological effect. Vitamin A, vitamin E, and carotenoids have demonstrated antioxidant, radical-quenching capabilities (McCay 1985; Surai and Speake 1998; Keys and Zimmerman 1999; Surai et al. 1999), and exposure of developing embryos to elevated levels of these antioxidants could be extremely beneficial, particularly immunologically, during such an oxidatively stressful time in development. Thus, the variable deposition of antioxidants into avian egg yolks may act as a mechanism by which females alter the quality of offspring.

A compensatory deposition strategy has three underlying assumptions: (1) attractive males must provide a better environment for growth and development than unattractive males (i.e., an environment that induces less oxidative stress), (2) that which is being allocated (in this case yolk antioxidants) must be a limited resource, and (3) the deposition of the resource must in some way mitigate the disadvantages experienced by an offspring sired by a lower-quality male (Navara et al. 2006*a*).

In the populations of house finches in eastern North America, more attractive males provision more, providing more direct benefits (Hill 1991, 2002), thus satisfying the first assumption of the compensatory deposition strategy. Second, the variation we see in the contents of eggs both within and among clutches, and the fact that females do not supply all eggs with equally high levels of antioxidants, suggests that antioxidants are limited resources. We found that females in better condition deposit more antioxidants into eggs, which is also suggestive of a cost associated with antioxidant deposition, and potentially satisfies the second assumption of the compensatory deposition strategy. If there were no cost associated with the deposition of yolk antioxidants, we would expect to see no relationship between female condition and yolk antioxidant concentration. A negative relationship between female condition and antioxidant concentration would indicate that females suffer some detriment related to the deposition of antioxidants, which is indicative of a cost. A positive relationship, which we found here, suggests that only females in the best condition can deposit maximal amounts of antioxidants into eggs, again suggesting that antioxidant deposition is costly. More work is needed, however, to determine more definitely whether a cost is associated with the deposition of antioxidants into yolk. Finally, the third assumption is met by the demonstrated immunoenhancing properties of antioxidants previously described (Bendich 1989; Chew 1993, 1996; McCay 1985; Keys and Zimmerman 1999).

House finches are socially monogamous, usually maintaining pair bonds through the breeding season. The DAH works only if females can hold back investment in a current reproductive attempt with a reasonable expectation of future reproductive attempts under different circumstances. For house finches, a species with short-lived individuals that face a high risk of death, a focus on immediate reproductive attempts may be the only viable strategy. By depositing antioxidants in a compensatory manner, females can maximize reproductive output from the current nesting effort.

The relevance of antioxidants in the yolks of eggs becomes even more interesting relative to patterns of the deposition of yolk testosterone in eggs of the same species. Total antioxidant concentrations were significantly positively correlated with total yolk androgen levels in our house finch eggs, and, like total yolk antioxidants, females deposited significantly more androgens into eggs sired by unattractive males (Navara et al. 2006*a*). Because testosterone is known to directly induce oxidative stress (von Schantz et al. 1999), females may deposit more antioxidants as a means of buffering the pro-oxidative effects associated with high levels of yolk androgens. Our results contrast those found in the lesser black-backed gull in which within-clutch variation in yolk testosterone was opposite that of yolk antioxidants (Royle et al. 2001). In line with our predictions, however, gull chicks hatching from eggs receiving both high testosterone and low antioxidant concentrations had the highest relative mortality rates, perhaps resulting from high levels of oxidative stress experienced by those chicks in relation to the contents of the eggs from which they hatched (Royle et al. 2001). Thus, females may deposit a combination of high androgen and antioxidant concentrations into eggs sired by less attractive males as a strategy to alter the condition of lower-quality offspring and compensate for detrimental conditions associated with a suboptimal mate.

Our study provides insight into the strategic basis behind a resource allocated to eggs before hatch and examines the relationships between the deposition of two physiologically relevant components in egg yolks. Our results suggest that the adaptive significance underlying the deposition of physiological resources may be dependent not only on surrounding social and environmental conditions but also on other critical components of the egg. Future studies measuring a multitude of egg contents, including a variety of hormones, antioxidants, critical nutrients, and antibodies, may provide additional insight into the level of control over offspring quality possessed by female birds. Additionally, the mechanism responsible for the variable deposition of antioxidants into egg yolks as well as the translation of visual stimuli into specific deposition strat-

egies is still unclear. Studies examining potential neurological pathways as well as changes in the function of transfer proteins regulating the manipulation of yolk content may be relevant steps toward uncovering the mechanism and the adaptive significance behind the deposition of yolk antioxidants.

Acknowledgments

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