Naturwissenschaften (2006) 93:597–602
DOI 10.1007/s00114-006-0146-6

SHORT COMMUNICATION

Carotenoid-dependent coloration of male American kestrels predicts ability to reduce parasitic infections

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Received: 23 February 2006 / Revised: 22 June 2006 / Accepted: 24 June 2006 / Published online: 16 August 2006

Abstract The signaling function of sexually selected traits, such as carotenoid-dependent avian plumage coloration, has received a great deal of recent attention especially with respect to parasitism and immunocompetence. We argue that parasite-mediated models of sexual selection may have an implicit temporal component that many researchers have ignored. For example, previous studies have demonstrated that carotenoid-dependent traits can signal past parasite exposure, current levels of parasitism, or the ability of individuals to manage parasitic infections in the future. We examined repeated measures of carotenoid-dependent skin color and blood parasitism in American kestrels (*Falco sparverius*) to distinguish whether coloration might signal current parasitism or the potential to deal with infections in the future. We found no evidence that coloration was related to current levels of parasitism in either sex. However, coloration of males significantly predicted their response to parasitism; males with bright orange coloration during prelaying, when mate choice is occurring, were more likely than dull yellow males to reduce their levels of infection by the time incubation began. Coloration during prelaying may advertise a male's health later in the breeding season. For kestrels, the ability to predict future health would be highly beneficial given the male's role in providing food to his mate and offspring. Coloration of females was not a significant predictor of parasitism in the future, and we provide several possible explanations for this result.

Keywords Sexual selection · American kestrels · *Falco sparverius* · Coloration · Parasitism · Carotenoids

Introduction

Hamilton and Zuk (1982) proposed that sexually selected traits may advertise heritable resistance to parasites, and females choosing bright or elaborately ornamented males as mates would secure resistance genes for their offspring. Much of the yellow, red, and orange coloration in birds that has evolved through sexual selection is based on carotenoid pigments (Gray 1996). Animals cannot produce these pigments, and so they are ultimately derived from the diet (Brush 1978). Carotenoids also have many biological functions, including roles in the immune system and as antioxidants for detoxifying free radicals (Olson and Owens 1998). This led Lozano (1994) to suggest a causal relationship between parasites and carotenoid-dependent color; while color would still signal individual quality, carotenoid-dependent signals need not be linked to genetic resistance to parasites as first envisioned by Hamilton and Zuk (1982). While there has been general support for the Hamilton–Zuk hypothesis (Hamilton and Poulin 1997; Møller et al. 2000), we believe that an important temporal component implicit in these types of hypotheses has been ignored (see also Hill and Farmer 2005). Traits such as bright coloration could potentially advertise past exposure to parasites, current health status, potential to resist parasitism in the future, or any combination of these, and previous studies have provided support for each possibility

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(e.g., Nolan et al. 1998; Brawner et al. 2000; Saks et al. 2003; Hill and Farmer 2005). Without question, a particular trait may convey information about all three of these characteristics, but it need not. In addition, many studies have failed to find the predicted relationships between parasitism and coloration (Hamilton and Poulin 1997), and one reason may be that temporal aspects have not been considered. We quantified both hematozoan parasitism and carotenoid-dependent coloration of American kestrels (*Falco sparverius*) multiple times during the breeding season, allowing us to evaluate potential temporal components of signals. Instead of feathers, we investigated skin color, which is dynamic and can change rapidly in response to a challenge (Faivre et al. 2003; McGraw and Ardia 2003). Unlike feathers, birds may be able to mobilize carotenoids from display tissues and divert them to their immune system (Faivre et al. 2003), and so these ornaments may provide reliable information on the potential of individuals to manage parasitic infections in the future. Kestrels have bare skin at the base of the bill and anterior to the eyes, the cere and lores, respectively, which ranges from dull yellow to bright orange (Bortolotti et al. 1996, 2003). The patterns of color suggest that they are sexually selected traits; they are more well developed (i.e., more orange) in males than females, and in sexually mature birds, and are most developed during mate choice (Bortolotti et al. 1996; Negro et al. 1998). Moreover, expression of color depends on carotenoids (this study; see also Bortolotti et al. 1996, 2003). We sampled color and parasite loads during the prelaying and incubation stages of the breeding season, and we tested whether current coloration was related to current levels of parasitism. We were also able to test whether color during prelaying could advertise a bird's ability to deal with its infection in the future. While coloration during mate choice may also advertise past exposure to parasites, it was not logistically possible to test this as it would require sampling birds either on migration or wintering area. We included both males and females in our analyses because evidence suggests that kestrels mate assortatively according to body condition and plumage quality (Bortolotti and Iko 1992; Bortolotti et al. 2002).

Materials and methods

We studied American kestrels breeding in nest boxes in north-central Saskatchewan, Canada, near Besnard Lake (55°N, 106°W) during 1994 and 1995. Kestrels arrived on our study area in mid- to late-April and began egg laying in mid-May. We captured kestrels using bal-chatri traps (Berger and Mueller 1959) during the prelaying period, and again by hand in nest boxes about 1 month later during incubation. For birds captured in both years, we used data only from the first capture. A drop of blood from each bird was used to make blood smears for determination of hematozoan infections (details in Dawson and Bortolotti 1999). We analyzed intensity of infection, measured as number of parasites detected in 100 microscope fields, and limited our analysis to birds infected with *Haemoproteus* as other parasite genera were relatively rare (Dawson and Bortolotti 1999). To meet assumptions of normality, these data were ln-transformed before analysis. The color of the cere and lores of each bird was scored on a 6-point scale using paint chips, with scores of 1 being dull yellow and 6 being bright orange, and these were summed to derive a total color score. Color determined using this system is significantly correlated with levels of carotenoids in the plasma of American kestrels (Bortolotti et al. 1996) and with measures of color determined objectively using digital photography (Bortolotti et al. 2003). We confirmed that color was carotenoid dependent by extracting carotenoids directly from cere tissues of 22 (10 females, 12 males) wild-strain kestrels kept in captivity during the breeding season at the University of Saskatchewan in 2000. Birds were euthanized as part of another investigation, and we immediately collected 5–39 mg of cere tissues from each bird for analysis. Carotenoids were extracted from tissues using methods modified from Surai and Speake (1998).

We used analysis of variance to describe how color scores varied between stages of the breeding season (prelaying and incubation), sex, and year. We also investigated color changes during the breeding season within individuals using paired *t* tests and correlation analysis. We tested whether parasite intensity was directly related to color scores at each stage of the breeding season using analysis of covariance. Color score was the dependent variable, while year and sex were the categorical variables, and parasite intensity was the covariate. Sampling birds during both prelaying and incubation allowed us to track variation in parasitism within individuals and examine whether color during prelaying could predict how birds dealt with their infections during the season. As most of our birds were infected by hematozoans (Dawson and Bortolotti 1999), it was not possible to compare birds that acquired infections to those that, for example, eliminated their infections during the breeding season. Instead, for this analysis, we simply classified birds into two groups based on whether their infections increased or decreased between prelaying and incubation. We then used logistic regression to test whether coloration during prelaying could predict a bird's potential, i.e., their ability to decrease their levels of parasitism. Our binary dependent variable was response (decrease/increase), while prelaying color score was the predictor variable. We also included sex and year as categorical covariates, as well as all possible interactions. For all analyses, we used a backward stepwise procedure to sequentially eliminate interactions and terms that were not significant. Final models always included the explanatory variables of interest, either color score or parasite intensity. Statistical tests were two-tailed, and we presented means ±1 standard error (SE) and considered the results significant at the 0.05 level.

Results

Previous evidence had suggested that color of the cere and lores of American kestrels was dependent on carotenoids. To confirm this, we extracted carotenoids directly from cere tissues and found significant relationships between cere color and total carotenoid concentration both when data for each sex were pooled (r=0.88, n=22, P<0.001) and when data were analyzed separately by sex (females: r=0.70, n=10, P=0.02; males: r=0.93, n=12, P<0.001).

Using data from 490 wild kestrels, randomly selecting one observation for those birds that were sampled during both prelaying and incubation, we found that males were more orange than females ($F_{1,484}$=384.39, P<0.0001), and birds were more orange during prelaying than incubation ($F_{1,484}$=21.07, P<0.0001; Fig. 1). In addition, we detected annual differences in color, with birds being more orange in 1995 than in 1994 ($F_{1,484}$=14.00, P<0.0001). Two interactions involving stage of breeding were also significant (season by sex: $F_{1,484}$=3.82, P=0.05; season by year $F_{1,484}$=9.79, P<0.01), so we analyzed color scores separately by year and sex. For females in both years and males in 1994, the color was significantly more orange in prelaying than in incubation (females 1994: $F_{1,135}$=17.44, P<0.0001; females 1995: $F_{1,135}$=4.70, P=0.03; males 1994: $F_{1,95}$=9.42, P<0.01); however, males in 1995 were similar in color between prelaying and incubation ($F_{1,117}$=0.27, P=0.60), accounting for the interactions we detected. Within individuals, the color was correlated between the two stages of the breeding season for females (r=0.42, n=74, P<0.0001; Fig. 2); males showed a similar but nonsignificant pattern (r=0.27, n=40, P=0.09). Paired t tests revealed that the color of individual females became less orange between prelaying and incubation (t=−7.27, df=73, P<0.001) but did not change appreciably in males (t=−0.60, df=39, P=0.55). Species composition of hematozoans, as well as prevalence and intensity of infections, in our kestrels has been reported elsewhere (Dawson and Bortolotti 1999). In this study, we limited our analyses to birds infected only

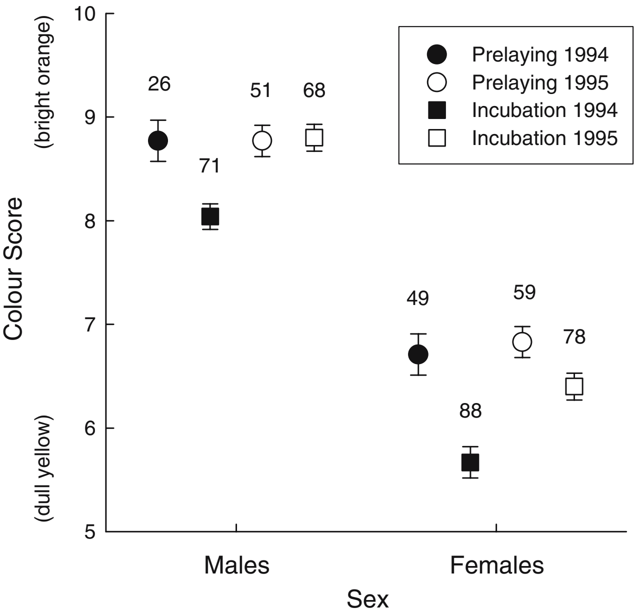

Fig. 1 Mean (±SE) coloration of the cere and lores of American kestrels during the prelaying and incubation periods. Coloration was scored on a 6-point scale, ranging from dull yellow (low scores) to bright orange (high scores), and the total score of each bird was subsequently calculated by summing the scores of the cere and lores. Sample sizes are given above error bars

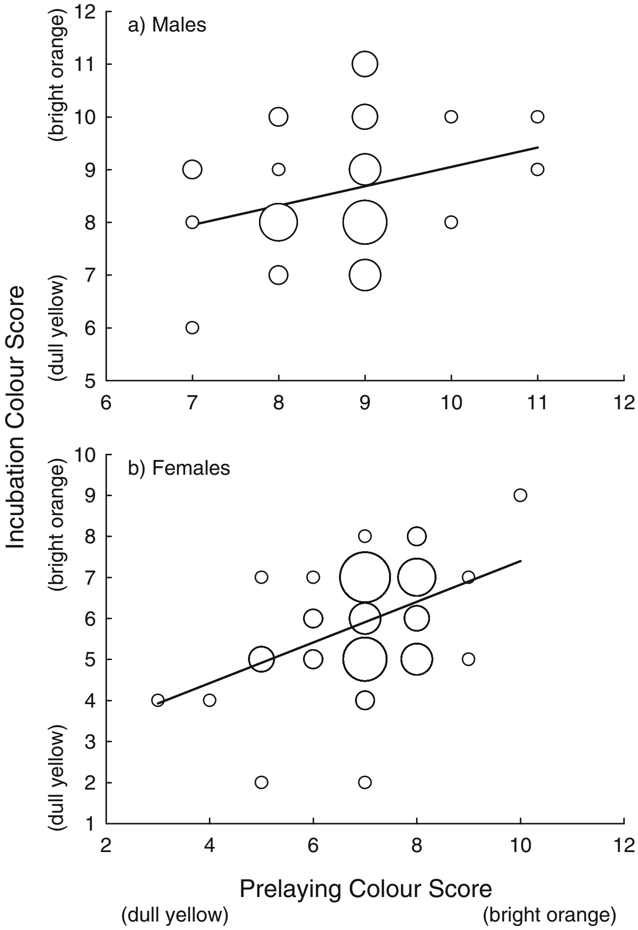

Fig. 2 Relationships between color scores of individual **a** male (P=0.09, n=40) and **b** female (P<0.0001, n=74) American kestrels during the prelaying and incubation periods. The relative number of data points at each potential combination of prelaying and incubation color scores is indicted by the relative size of the symbol

with species of *Haemoproteus* (*Haemoproteus tinnunculi* and *Haemoproteus brachiatus*), both by themselves and in mixed infections. Males had intensities of 87.5±18.8 parasites/100 fields (n=62) during prelaying and 71.7±6.7 parasites/100 fields (n=117) during incubation. Females during prelaying averaged 168.2±35.6 parasites/100 fields (n=100) and 69.8±6.0 parasites/100 fields (n=148) during incubation.

After controlling for significant sex differences in coloration during prelaying ($F_{1,158}$=137.99, P<0.0001), we could find no influence of parasite intensity on coloration ($F_{1,158}$=1.50, P=0.22). Similarly, there was no effect of parasite intensity on color during incubation ($F_{1,259}$=0.02, P=0.88), although males were more orange than females ($F_{1,259}$=240.57, P<0.0001), and birds were more orange during 1995 than 1994 ($F_{1,259}$=33.13, P<0.0001). Females classified as having levels of infection increase between prelaying and incubation had average increases of 70.0±10.2 (n=26), while males in this group increased by 80.2±18.9 (n=9) parasites. Females classified as reducing their infections over this time period decreased their levels of infection by 103.6±28.9 (n=21) parasites, while males decreased by 114.5±52.4 (n=19) parasites. Logistic regression suggested that prelaying birds with more orange coloration were better able to deal with their infections because they were more likely to have their levels of infection decrease between prelaying and incubation (χ^2=5.01, df=1, P=0.02). The effect of sex (χ^2=4.77, df=1, P=0.03), as well as the interaction between sex and color (χ^2=5.36, df=1, P=0.02), were also significant. These latter two results in the initial model indicated that relationships between color and ability to deal with parasitism were different for males and females, so we also analyzed data separately for each sex. For females, there was no effect of coloration during prelaying on their ability to deal with parasites (χ^2=0.40, df=1, P=0.53; Fig. 3). The probability of males having their intensity of infection decrease during the breeding season increased as their prelaying color became more orange (χ^2=5.00, df=1, P=0.03; Fig. 3). This suggests that coloration of male kestrels during prelaying could predict their potential with regard to parasitism in the future.

Discussion

Carotenoid-dependent coloration in some species of birds is correlated with current levels of hematozoan parasitism (e.g., Merilä et al. 1999; Hõrak et al. 2001). Our results showed that while color of the cere and lores of American kestrels was not related to current levels of hematozoan parasitism, coloration predicted the potential of males to manage their infections. Males with bright orange coloration during the prelaying period were more likely than males with less orange color to decrease their levels of infection by the time incubation began (Fig. 3). Color may therefore not reflect resistance to becoming infected but instead may predict the ability of an individual to recover from infection (Hill and Farmer 2005). Similarly, plumage color has been shown to predict the potential of greenfinches (*Carduelis chloris*); bright birds were able to clear experimentally administered virus loads faster than dull birds, had lower infection intensities, and tended to produce more antibodies (Lindström and Lundström 2000). House finches (*Carpodacus mexicanus*) with redder plumage were also able to recover faster than birds with yellower plumage from experimental challenges with *Mycoplasma* bacteria (Hill and Farmer 2005). McGraw and Ardia (2003) showed that bill color and plasma carotenoids of zebra finches (*Taeniopygia guttata*) changed in response to challenge with phytohemagglutinin, suggesting that carotenoids from these tissues may have been used directly by the immune system. A similar process may be operating in male kestrels. If carotenoids in the cere and lores can be mobilized by kestrels, then coloration may signal their ability to respond to infections, as originally speculated by Lozano (1994). While individuals may trade-off carotenoids by immobilizing them in signals such as feathers at the expense of immune defense (Olson and Owens 1998), such a trade-off may not exist for coloration of bare patches of skin. The color of the cere and lores in kestrels, and the bill color in other species, may therefore be a particularly revealing signal for advertising future ability to deal with parasites.

It would be important for female kestrels to evaluate potential of their mates before egg laying. Male kestrels are the primary providers of food from the start of the breeding

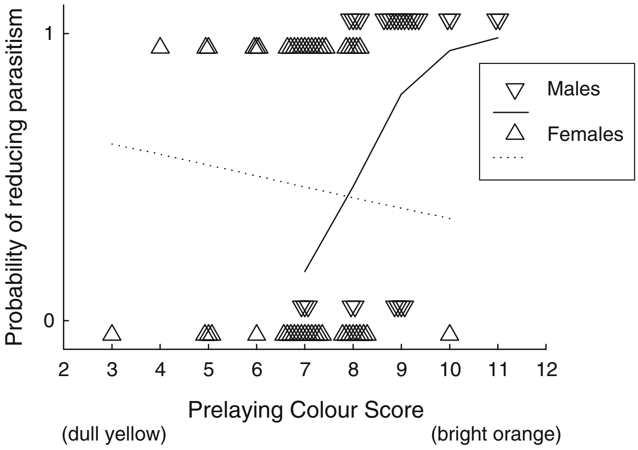

Fig. 3 The probability of American kestrels having their intensity of infection increase (0) or decrease (1) over the breeding season in relation to coloration of their cere and lores during the prelaying period. Prediction lines are derived from logistic regressions. Data points have been offset slightly from their actual values to illustrate their distribution on the plot

episode until nestlings are about 10 days old (personal observation; Balgooyen 1976), a period of approximately 50 days. We have also shown previously that male kestrels with high intensity hematozoan infections during incubation are in poor body condition (Dawson and Bortolotti 2000) and may therefore be less able to adequately provide for their mates and offspring. Females may also benefit from choosing males with the potential to reduce parasite loads because there is less risk of parasites being transmitted from the male to both the female herself as well as the offspring, both of which could have detrimental consequences for survival (Dawson and Bortolotti 2000; Hõrak et al. 2001). While hematozoan parasites are often thought to be relatively benign, both observational and experimental studies have shown that these parasites can be pathogenic (e.g., Dawson and Bortolotti 2000, 2001; Merino et al. 2000; Garvin et al. 2003).

Females are often deemed to be the choosier sex; however, given the large investment in reproduction by male kestrels (Balgooyen 1976), males should also exercise mate choice (Burley 1977). In fact, there is evidence for assortative mating in our population of kestrels with respect to both body condition and feather quality (Bortolotti and Iko 1992; Bortolotti et al. 2002). It was therefore somewhat surprising that coloration of female kestrels was not predictive of potential to manage parasite loads, especially given that females with intense infections appear to have reduced survival (Dawson and Bortolotti 2000). There are several potential explanations to account for this. First, if there is selection for a particular trait in males, the trait may also be present in females because fathers will also transmit genes to their daughters (Lande 1980). Hence, females may have colorful ceres and lores due to a genetic correlation and not because these structures have any signaling function. Alternatively, coloration of females may advertise some other aspects of quality. It is clear that coloration of females becomes less orange, fading to dull yellow, as the breeding season progresses (Fig. 1; see also Negro et al. 1998), and these changes in coloration may be due to females depositing carotenoids in reproductive tissue and eggs (Negro et al. 1998). This may be particularly important because hatching is a form of oxidative stress, and so antioxidants such as carotenoids would be particularly valuable (Surai 1999). If female color is important for males, then it may advertise a female's potential to hatch viable offspring, but more studies are needed to evaluate this argument. Indeed, Shykoff and Widmer (1996) suggested that relationships, or lack thereof, between parasitism and carotenoid-dependent signals depend on the carotenoid allocation strategy of the individual as well as the availability of carotenoids. They outlined scenarios where both positive and negative relationships, as well as no relationship, between carotenoid signals and parasitism would be expected. More research is needed to fully understand how individual birds allocate these pigments among display traits and their immune systems.

Acknowledgments We thank the many people who helped sample birds, and the late G. Bennett who examined smears for parasites. We thank E. Korpimäki, D. Shutler, and J. Wiehn for comments on various drafts of the manuscript. Funding was provided by the Natural Sciences and Engineering Research Council of Canada through a grant to GRB and a scholarship to RDD. The Canadian Wildlife Federation and Northern Scientific Training Program also provided funding to RDD. Our protocol was approved by the University of Saskatchewan Animal Care Committee.

References

Balgooyen TG (1976) Behavior and ecology of the American kestrel (*Falco sparverius* L.) in the Sierra Nevada of California. Univ Calif Publ Zool 103:1–85

Berger DD, Mueller HC (1959) The bal-chatri: a trap for the birds of prey. Bird-Banding 30:18–26

Bortolotti GR, Iko WM (1992) Non-random pairing in American kestrels: mate choice versus intrasexual competition. Anim Behav 44:811–821

Bortolotti GR, Negro JJ, Tella JL, Marchant TA, Bird DM (1996) Sexual dichromatism in birds independent of diet, parasites and androgens. Proc R Soc Lond B Biol Sci 263:1171–1176

Bortolotti GR, Dawson RD, Murza GL (2002) Stress during feather development predicts fitness potential. J Anim Ecol 71:333–342

Bortolotti GR, Fernie KJ, Smits JE (2003) Carotenoid concentration and coloration of American kestrels (*Falco sparverius*) disrupted by experimental exposure to PBBs. Funct Ecol 17:651–657

Brawner WR, Hill GE, Sundermann CA (2000) Effects of coccidial and mycoplasmal infections on carotenoid-based plumage pigmentation in male house finches. Auk 117:952–963

Brush AH (1978) Avian pigmentation. In: Brush AH (ed) Chemical zoology, vol 10. Aves. Academic, New York, pp 141–164

Burley N (1977) Parental investment, mate choice, and mate quality. Proc Natl Acad Sci USA 74:3476–3479

Dawson RD, Bortolotti GR (1999) Prevalence and intensity of hematozoan infections in a population of American kestrels. Can J Zool 77:162–170

Dawson RD, Bortolotti GR (2000) Effects of hematozoan parasites on condition and return rates of American kestrels. Auk 117:373–380

Dawson RD, Bortolotti GR (2001) Sex-specific associations between reproductive output and hematozoan parasites of American kestrels. Oecologia 126:193–200

Faivre B, Grégoire A, Préault M, Cézilly F, Sorci G (2003) Immune activation rapidly mirrored in a secondary sexual trait. Science 300:103

Garvin MC, Homer BL, Greiner EC (2003) Pathogenicity of *Haemoproteus danilewskyi*, Kruse, 1890, in blue jays (*Cyanocitta cristata*). J Wildl Dis 39:161–169

Gray DA (1996) Carotenoids and sexual dichromatism in North American passerine birds. Am Nat 148:453–480

Hamilton WD, Zuk M (1982) Heritable true fitness and bright birds: a role for parasites? Science 218:384–387

Hamilton WJ, Poulin R (1997) The Hamilton and Zuk hypothesis revisited: a meta-analytical approach. Behaviour 134:299–320

Hill GE, Farmer KL (2005) Carotenoid-based plumage coloration predicts resistance to a novel parasite in the house finch. Naturwissenschaften 92:30–34

Hõrak P, Ots I, Vellau H, Spottiswoode C, Møller AP (2001) Carotenoid-based plumage coloration reflects hemoparasite infection and local survival in breeding great tits. Oecologia 126:166–173

Lande R (1980) Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution 34:292–305

Lindström K, Lundström J (2000) Male greenfinches (*Carduelis chloris*) with brighter ornaments have higher virus infection clearance rate. Behav Ecol Sociobiol 48:44–51

Lozano GA (1994) Carotenoids, parasites, and sexual selection. Oikos 70:309–311

McGraw KJ, Ardia DR (2003) Carotenoids, immunocompetence, and the information content of sexual colors: an experimental test. Am Nat 162:704–712

Merilä J, Sheldon BC, Lindström K (1999) Plumage brightness in relation to haematozoan infections in the greenfinch *Carduelis chloris*: bright males are a good bet. Ecoscience 6:12–18

Merino S, Moreno J, Sanz JJ, Arriero E (2000) Are avian blood parasites pathogenic in the wild? A medication experiment in blue tits (*Parus caeruleus*). Proc R Soc Lond B Biol Sci 267:2507–2510

Møller AP, Biard C, Blount JD, Houston DC, Ninni P, Saino N, Surai PF (2000) Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability? Avian Poult Biol Rev 11:137–159

Negro JJ, Bortolotti GR, Tella JL, Fernie KJ, Bird DM (1998) Regulation of integumentary colour and plasma carotenoids in American Kestrels consistent with sexual selection theory. Funct Ecol 12:307–312

Nolan PM, Hill GE, Stoehr AM (1998) Sex, size, and plumage redness predict house finch survival in an epidemic. Proc R Soc Lond B Biol Sci 265:961–965

Olson VA, Owens IPF (1998) Costly sexual signals: are carotenoids rare, risky or required? Trends Ecol Evol 13:510–514

Saks L, Ots I, Hõrak P (2003) Carotenoid-based plumage coloration of male greenfinches reflects health and immunocompetence. Oecologia 134:301–307

Shykoff JA, Widmer A (1996) Parasites and carotenoid-based signal intensity: how general should the relationship be? Naturwissenschaften 83:113–121

Surai PF, Speake BK (1998) Distribution of carotenoids from the yolk to the tissues of the chick embryo. J Nut Biochem 9:645–651

Surai PF (1999) Vitamin E in avian reproduction. Poult Avian Biol Rev 10:1–60