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Carotenoids and life-history evolution in animals

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Abstract

Animals must allocate finite resources amongst competing demands. A suite of such trade-offs is thought to occur in the deployment of carotenoids, being widely responsible for sexual coloration and also important in antioxidant and immune defences. Experimental manipulation of dietary carotenoid availability is a useful approach for elucidating the mechanistic bases of carotenoid allocation trade-offs. Recent work using birds has shown that both sexual display and immune defences can be limited by carotenoid availability, providing support for the hypothesis that males allocating greater amounts of carotenoids to sexual coloration are advertising their superior health. Carotenoid availability has also been shown to limit egg-laying capacity in birds, although it remains to be seen whether carotenoid display in females advertises reproductive potential. More experiments are required to ascertain the importance of direct (material) and indirect (genetic) benefits accruing through choosing to mate with individuals that have greater carotenoid display.

Keywords: Antioxidant; Carotenoid; Egg production; Immune function; Sexual signal; Trade-off

Animals have limited amounts of resources available, which must be allocated amongst different body systems. Where two or more such systems utilise the same resource, trade-offs can arise, whereby increased allocation to one activity results in decreased resource availability for alternative uses. For example, increased investment in reproduction may trade against survival, whilst increased investment in gamete quality may trade against gamete number. Under such circumstances evolution will favour individuals having the capacity to deploy resources in a way that maximises their survival and reproductive success. This concept of trade-offs is a cornerstone of life-history theory, the study of what causes differences in evolutionary fitness amongst alternate life-history strategies [1]. Over the last 10 years, a suite of such trade-offs has been hypothesised to occur in the deployment of dietary carotenoids in animals [2–5], which are widely responsible for sexual and other coloration, and are also important in both antioxidant and immune defences [6,7]. However, until recently there have been few direct tests of whether carotenoid availability can be limiting for reproduction, antioxidant and immune defences in wild animals, underlying physiological trade-offs between such activities and sexual display.

Mate choice studies in birds and fish have shown that the most preferred individuals are often those expressing greater carotenoid pigmentation in sexual signals (female choice amongst males: e.g. [8,9] and male choice amongst females: e.g. [10,11]). But what aspects of individual quality are revealed by such signals, or put differently, what is to be gained by choosing to mate with an individual who has a greater quantity of carotenoids in sexual display? Evolutionary theory posits that visual signals must be costly to produce, in order to enforce honesty in signalling [12,13]. It was originally proposed that superior carotenoid display could advertise an individual's ability to forage for carotenoids, which might be scarce in the environment and difficult to acquire in sufficient quantities in the diet [14,9]. Subsequently, since certain endoparasites (coccidia) can impair intestinal absorptive function (e.g. [15]), and

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because certain ectoparasites can directly conceal or damage carotenoid display in integument it was suggested that superior carotenoid display might reveal a low parasite burden [16,17].

Experiments have confirmed that both dietary carotenoid availability [17,18] and gut parasitism [19] can limit the expression of sexual coloration. However, whether differences in foraging efficiency and gut function provide a sufficient explanation for the information content of carotenoid-based sexual display has been questioned recently [2–7]. Specifically, it has been hypothesised that trade-offs can exist in carotenoid allocation between maintaining sexual display and health: males in better condition should require fewer carotenoids for antioxidant [3] and immune defences [2] and could therefore utilise more of this resource to enhance sexual display, thereby advertising their superior health [6,7]. This has recently been extended to encompass the idea that carotenoid display could reveal an individual's capacity to produce high quality eggs or sperm, due to the role of carotenoids and other antioxidants in the production and antioxidant protection of gametes [4,5].

There is a considerable amount of correlative evidence suggesting carotenoid-limitation of reproduction and immunocompetence, and links between the expression of carotenoid-based sexual display and other life-history parameters; this has been reviewed comprehensively elsewhere [6,7]. In this article, I will consider those studies where carotenoid availability has been experimentally manipulated through dietary supplementation to tease out the mechanistic bases of carotenoid allocation trade-offs. The work that I shall refer to relates to birds, which are excellent models for studying such resource allocation trade-offs because carotenoids are known to play important roles in determining sexual attractiveness [2–7] and egg quality in avian taxa [4].

Carotenoids, immunocompetence and sexual display

In work using captive male zebra finches *Taeniopygia guttata* it has recently been shown that dietary supplementation with a combination of lutein and zeaxanthin resulted in increased bill redness (a sexual signal) and sexual attractiveness [20]. This study also assessed the cell-mediated immune responses of males by measuring the swelling of the propatagium (a flap of skin at the leading edge of the wing) following intradermal injection with phytohemagglutinin (PHA),[1] which has a mitogenic effect on T-lymphocytes. Carotenoid-supplemented males had significantly larger PHA responses compared to controls [20]. A separate study of European blackbirds *Turdus merula* has revealed that intraperitoneal injection with sheep red blood cells (to raise a humoral immune response) caused a decline in bill color (a sexual signal), suggesting that carotenoids were reallocated from the bill to circulation to be used during immune activation [21]. Similar findings have recently been reported in another study of zebra finches [22]. Together, these studies provide the first direct support for the idea that sexual attractiveness and immune defences can both be limited by, and compete for access to, carotenoid pigments. Interestingly, the red color of the bill in male zebra finches is thought to be produced by ketocarotenoids, which are absent in the diet and therefore must be the result of metabolic transformations of ingested carotenoids [23]. Such transformations are inefficient and may be energetically costly, suggesting a further possible mechanism by which carotenoid-based sexual signals could reveal individual quality [24]. In theory, if carotenoid display reveals health, then the specific types of carotenoids used in sexual signals should be those that are most limiting for antioxidant and immune defences. However, this remains to be investigated.

Contrasting results have recently been provided by Navara and Hill [25], who found that whilst dietary supplementation with a combination of lutein and zeaxanthin resulted in greater carotenoid display in captive male American goldfinches *Carduelis tristis*, the treatment did not influence cell-mediated or humoral immune responses, or resistance to ocular infection with *Mycoplasma gallisepticum*. One possible explanation for the contrasting conclusions reached by these different studies [20–22,25] is that the scale of immune defences, and hence the requirement for carotenoids, differs amongst species. Birds exhibit considerable interspecific variation in basal leukocyte counts, and in the sizes of immune organs and immune responses following experimental challenge, which has been linked to the level of exposure to, and hence coevolution with, virulent parasites (e.g. [26,27]). Blood carotenoid levels in birds are often considerably higher than in mammals, which has led to the suggestion that birds' immune systems are unlikely to be carotenoid-limited [28]. Recent work clearly shows that this is not a general rule amongst species [20–22]. However, an interesting direction for further work will be to explore whether differences in exposure to parasites/diseases, or to reactive oxygen species (ROS) (as influenced by work rate, for example), can explain patterns of interspecific variation in blood carotenoid levels and susceptibility to carotenoid-limitation of immune function. At present it is not possible to rule out the explanation that methodological differences amongst studies [20–22,25] account for their contrasting results. In particular, the doses of carotenoid supplements differed greatly between studies, being $11\,\mu\mathrm{g\,mL}^{-1}$ drinking water for 5 weeks [22] and $50\,\mu\mathrm{g\,mL}^{-1}$ drinking water for 8 weeks [20], respectively, in the two independent studies of zebra finches, and ei-

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[1] *Abbreviations used:* ROS, reactive oxygen species; PHA, phytohaemagglutinin; VLDL, very low-density lipoprotein.

ther 10 (low treatment), 100 (medium) or 1000 $\mu g\,mL^{-1}$ drinking water (high) for 3 months in the study of American goldfinches [25]. It seems possible that in Navara and Hill's study [25], the medium and high doses elevated body carotenoid levels to an extent that the carotenoids lost their effectiveness as antioxidants [29]. Little is known of what quantities of carotenoids are consumed by birds in their natural diets, and average blood carotenoid concentrations in free-living individuals have been determined for relatively few species [30–35], so deciding appropriate doses to use in supplemental feeding studies is difficult. It would be advantageous to carry out similar studies using free-living birds, for which variation in both natural and experimentally elevated blood carotenoid levels can be measured, and supplemental carotenoid doses decided accordingly.

So there is now some evidence suggesting that females choosing to mate with males having greater carotenoid display stand to obtain a mate who can mount stronger immune responses [20,22]. However, it remains unknown whether such mate preferences translate into direct benefits to females, such as a greater male contribution to the care of offspring or a reduced risk that he will transmit infectious diseases [2]. This could be investigated experimentally by supplementing the diet of free-living males with carotenoids, then measuring the effects on their work rate during chick rearing, and their burden of parasites and diseases and the effects on infection in females. There is also the intriguing possibility that choosy females may obtain genes for viability, that confer offspring with enhanced efficiency in resistance to parasites, diseases [36], and oxidative stress [3]. It has been shown that brother-pairs of zebra finches, wherein each duo one male was fed a control diet (low carotenoid intake) and its brother was fed the same diet supplemented with additional carotenoids (lutein and zeaxanthin), do not have inherently similar plasma carotenoid concentrations suggesting variation in carotenoid uptake from the diet is not strongly influenced by genetic (or maternal) effects [20]. However, genes may shape other stages of the carotenoid acquisition and utilization process, such as an individual's foraging or immune efficiency [20]. It has been established using birds that paternal genetic effects on offspring performance can be predicted by the expression of secondary sexual traits (e.g. [37]). However, it is not known whether male carotenoid display, as a specific case of sexual ornamentation, reveals the capacity to provide females with 'good genes' for offspring. This is an interesting possibility because of the role of carotenoids in activating gene expression [38].

Carotenoids, egg-laying capacity and sexual display

Egg-removal experiments in wild birds, where the first-laid (or more) eggs are taken from the nest to induce production of an enlarged clutch size, have revealed that females incur considerable costs solely through egg production, including increased parasitism [39], and reduced chick-rearing capacity [40], and adult survival [41]. The same experimental protocol has also been found to reduce the quality of the eggs themselves (i.e., chick survival) [42]. However, there have not been many studies on the physiological mechanisms underlying such effects [43]. It was recently hypothesised that carotenoid-limitation may explain such costs of egg production [4]. Work on domestic hens has suggested that an important function of maternally derived yolk carotenoids is to protect yolk lipids against ROS. Egg yolk largely consists of highly unsaturated fatty acids that nourish the developing embryo, but render embryo tissues vulnerable to attack by ROS [4]. The risk of lipid peroxidation is likely to be promoted by the high rates of oxidative metabolism displayed in embryos, and to increase as embryogenesis proceeds because of the accelerating rate of oxygen diffusion through the shell to fuel metabolism [4]. Chicks may be especially vulnerable to oxidative stress because the process of hatching incurs sudden exposure to atmospheric concentrations of oxygen, and hatchlings then undergo a dramatic increase in metabolic rate with the onset of pulmonary respiration and post-hatching growth [4]. Studies of domestic hens have shown that supplementation of the maternal diet with carotenoids (β-carotene [44] and combination of lutein, citranaxanthin, canthaxanthin, and β-apo-8′-carotenoic acid and [45]) results in carotenoid-enrichment of egg yolk and chick tissues, and reduces tissue susceptibility to lipid peroxidation [45] and oxidative stress in vitro (astaxanthin [46]).

But in nature, can carotenoid availability limit a female's capacity to lay? This question has recently been addressed using free-living lesser black-backed gulls *Larus fuscus*, in which dietary supplementation with a combination of lutein, zeaxanthin, canthaxanthin, and β-carotene was found to increase plasma carotenoid concentrations and antioxidant activity in vitro [35], but did not influence the proportion of females that laid, or the timing, size or mass of first clutches [35,47,48]. However, carotenoid-fed females produced eggs with reduced susceptibility to lipid peroxidation in vitro, suggesting that carotenoid availability can limit egg quality [47]. Moreover, carotenoid supplementation markedly increased the capacity of gulls to re-lay following removal of first clutches: one-third more carotenoid-fed females re-laid compared to controls [48]. Since clutch loss through predation is common, carotenoid-limitation of egg-laying capacity could be an important constraint on reproductive performance in wild birds [48]. The mechanism by which carotenoid availability influences egg-laying capacity may relate to yolk synthesis.

Carotenoids and retinoic acid can trigger the expression of estrogenic enzymes, which have been shown to increase oestrogen production in vitro [49,50]. Oestrogen stimulates hepatic expression of the genes that code for synthesis of very low-density lipoprotein (VLDL) and vitellogenin, the main sources of yolk lipid and protein, respectively [51]. A non-mutually exclusive possible explanation is that supplemental carotenoids could have enhanced the antioxidant protection of circulating VLDL and vitellogenin [48].

Individual carotenoids are selectively transferred from the yolk to specific tissues in the embryo, suggesting that they serve particular roles during offspring development [52]. This raises the question of whether there is a 'recipe' for a good egg, in terms of the particular balance of carotenoids deposited into yolk, and whether this varies amongst species. A study of wild lesser black-backed gulls has shown that supplementation of the maternal diet with a cocktail of four carotenoids (lutein, zeaxanthin, canthaxanthin, and β-carotene) resulted in increased yolk concentrations of at least eight individual carotenoids (lutein, *cis*-lutein, zeaxanthin, canthaxanthin, β-cryptoxanthin, echinenone, and β-carotene), and the percentage profile of yolk carotenoids was positively correlated between supplemented and control (non-supplemented) groups [47]. This suggests that females must metabolically transform dietary carotenoids and differentially transfer specific carotenoids into yolk, which could represent a cost of reproduction [47]. However, the significance of particular yolk carotenoid profiles for offspring awaits study.

Evidence is accumulating that yolk carotenoid levels vary markedly amongst bird species, both in terms of mean concentrations for species and the degree of intraspecific variation (Table 1). However, it remains unclear the extent to which such variation reflects differences amongst species in carotenoid-limitation of egg-laying capacity, or the requirements for carotenoids in embryos and hatchlings. It has been suggested that factors including the developmental mode of offspring and concomitant risk of oxidative stress during embryogenesis could determine the requirement for yolk carotenoids, precocial species that hatch in a relatively advanced state of development having a greater need for yolk carotenoids compared to altricial species that are naked and blind at hatching [4]. There is much scope for phylogenetic comparative analyses across species, to investigate patterns of covariation amongst yolk carotenoid profiles and life-history parameters in adults and offspring.

Females often bear extravagant, secondary sexual ornaments such as carotenoid display. The original assumption that such ornaments in females are a non-functional, genetically correlated result of sexual selection on males has been questioned recently [57]. It has been hypothesised that females may advertise their egg-laying capacity to prospective mates through the expression of carotenoid display [4]. In two-spotted gobies, *Gobiusculus flavescens*, a small marine fish species, males prefer to mate with females having the brightest orange-red abdomen—a signal thought to reveal the carotenoid content of eggs that are visible through the skin [58]. However, it has yet to be confirmed that reproductive success covaries with egg carotenoid content in this species. Similarly, work on house finches *Carpodacus mexicanus* has shown that females with the most intense carotenoid plumage display are preferred as mates. However, there was no relationship between female coloration and reproductive success [59]. In lesser black-backed gulls, females with duller carotenoid-based integument coloration (bill, bill spot, eye ring, gape flange, and legs) produce clutches that exhibit a greater decline in yolk carotenoid levels over the laying sequence [35]. However, it is not known whether female gulls with greater carotenoid coloration are preferred as mates. Therefore, it remains unclear whether carotenoid display in females reveals reproductive potential and egg-laying capacity specifically. This would be an interesting direction for further work.

Table 1
Concentrations of carotenoids in the egg yolk of free-living birds

Species	Total carotenoids ($\mu g\,g^{-1}$ yolk) mean (SD) *n*	Coefficient of variation (%)	Reference
Emperor penguin, *Aptenodytes forsteri*	8.60 (*) 6	—	[53]
Lesser black-backed gull, *Larus fuscus*	71.60 (29.06) 20	40.59	[52]
Common moorhen, *Gallinula chloropus*	47.50 (21.80) 10	45.90	[52]
American coot, *Fulica americana*	131.00 (18.96) 10	14.47	[52]
Northern gannet, *Morus bassanus*	17.70 (3.70) 4	20.90	[54]
Great skua, *Catharacta skua*	12.70 (6.30) 5	49.61	[54]
American white pelican, *Pelecanus erythrorhynchos*	150.90 (67.80) 8	44.93	[54]
Double-crested cormorant, *Phalacrocorax auritus*	115.70 (79.80) 21	68.97	[54]
Canada goose, *Branta canadensis*	22.10 (3.14) 5	14.21	[55]
Barn swallow, *Hirundo rustica*	19.00† (*) 18	—	[56]

* Value not given in primary reference.

† Value estimated from figure in primary reference.

Conclusions

Important life-history components in animals can clearly be limited by carotenoid availability, including the expression of carotenoid display and therefore sexual attractiveness, and also egg-laying capacity in birds. Moreover, there is increasing evidence of the existence of trade-offs in carotenoid allocation between secondary sexual ornaments and immune defence, providing us with insights into the likely information content of carotenoid-based sexual display. However, the relative importance of direct (material) and indirect (genetic) benefits accruing to females choosing to mate with more carotenoid-rich males requires further study. It also remains unclear what aspects of individual quality may be signalled by carotenoid display in females. Since carotenoid availability can constrain the capacity of females to lay per se, and the quality of eggs, it would be interesting to know whether carotenoid display can trade against egg-laying capacity. Other life-history parameters that have been hypothesised to be potentially carotenoid-limited, and to be revealed by carotenoid display, include sperm quality and work capacity. However, empirical tests of these hypotheses are lacking at present. A consideration of the ecological and evolutionary implications of non-antioxidant functions of carotenoids and their metabolites would also be timely, including for example intercellular signalling, gene expression, and regulation of enzyme activity [38]. Such functions could affect an individual's health in more subtle ways than ecologists have so far attempted to measure. In all these contexts there is a particular need for experimental studies of free-living animals, because such conditions provide the best scope for testing the importance of carotenoid availability in shaping life-history patterns.

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