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Carotenoid-dependent Signals: Indicators of Foraging Efficiency, Immunocompetence or Detoxification Ability?

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ABSTRACT

Carotenoids are a large group of more than 600 different biochemicals synthesized by bacteria, fungi, algae and plants. Higher animals obtain these biologically active pigments in the diet, and they are subsequently used in diverse bodily functions. They are disproportionately common components of the color of animal signals such as those used in sexual communication, signaling between offspring and their parents and in warning colors. Carotenoids also play important roles in various aspects of immune function and detoxification, and a trade-off between signaling and these physiological functions has been hypothesized. They may either signal foraging efficiency, immunocompetence or anti-oxidative potential of signalers. Carotenoids are usually limiting the maximum possible expression of a signal since supplementation experiments have commonly produced more exaggerated signals. There is considerable evidence supporting the prediction that parasites reduce the expression of carotenoid-based signals, and that such signals reliably reflect the ability of the signaler to produce a strong immune response. There is also some evidence consistent with the hypothesis that signals may reflect the anti-oxidant potential of signalers. If carotenoids commonly have important physiological functions among captive and free-living organisms, we hypothesize several ways in which this should affect the design of animal breeding programs and conservation programs involving captive breeding.

Keywords: Detoxification, immune function, limitation of carotenoids, offspring signals, parasitism, sexual selection

1. INTRODUCTION

Signals are actions or structures that increase the fitness of an individual by altering the behavior of other organisms detecting it, and that have characteristics that have evolved because they have an effect. Signals are produced by a sender, and intended and unintended receivers may perceive this signal and decode its message. Signaling contexts vary dramatically, including attraction between individuals of different interests such as sexual signals among males and between males and females, or between offspring and their parents, or they may be antagonistic such as those between prey and predators or between hosts and their parasites. A surprisingly large number of signals are carotenoid-based, although carotenoids may not always be in ready supply because they must be ingested by most organisms and often undergo metabolic transformations before being deposited in signals. Why should it be the case that such substances are so common in many different kinds of signaling contexts?

Carotenoids are a group of more than 600 different biochemicals with similar biochemical properties. They can be grouped into xanthophylls and carotenes based

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on their molecular structure, *i.e.* oxygenated or aliphatic chains. Carotenoids can only be synthesized by algae, bacteria, fungi and plants (Fox, 1979; Brush, 1981; Goodwin, 1984; Latscha, 1990; Stradi, 1998), and they hence must be ingested by higher animals in order to be used in physiological processes or displayed in signals. Animals differ in their rate of carotenoid absorption (Scheidt, 1989), but also in their ability to convert ingested carotenoids into other pigments (Fox, 1979; Goodwin, 1984; Putnam, 1992; Stradi, 1998), and their rate of carotenoid deposition in signals (Brush and Power, 1976; Hudon, 1991). Birds of the species studied so far preferentially accumulate xanthophylls such as lutein, zeaxanthin and canthaxanthin, but less readily accumulate carotenes, such as β-carotene (Brush, 1981; Goodwin, 1984; Stradi, 1998; Royle *et al.*, 1999). A large number of different carotenoids have been found in feathers of birds, some ingested and others derived from oxidative transformation of other carotenoids. For example β-carotene may be converted into echinenone, and sometimes further transformed into canthaxanthin or 3-hydroxy-echinenone, adonirubin and astaxanthin (Figure 1; Stradi, 1998). In other species, there is apparently little evidence of transformation of carotenoids from the diet to deposition in tissue (Partali *et al.*, 1987). Furthermore, β-carotene can be converted into vitamin A by birds (Damron *et al.*, 1984; Schaffer *et al.*, 1988), and vitamin A also has antioxidant activity. Thus xanthophylls in signals may be of dietary origin, or they may arise through transformation of other xanthophylls or carotenes (Stradi, 1998). Xanthophylls are a much more widely distributed, plentiful and diverse group of compounds than carotenes (Latscha, 1990). Thus xanthophylls in circulation and storage seem more likely to be of direct dietary origin than arising through transformation of carotenes. So the occurrence of any one xanthophyll in a signal might reveal availability of donor xanthophyll(s) or carotene(s), and hence reflect their allocation to signals rather than physiological processes such as immune function and anti-oxidant activity.

Why are carotenoid-based signals so common? Latscha (1990) has suggested that carotenoids are the most widely distributed pigments in nature, not just in animals bearing signals, but also in food items. This fact may render them common in signals, although this explanation does not easily account for sex- and age-differences in the presence and the intensity of carotenoid-based signals. What do carotenoid-based signals reveal about the signaler? Is it foraging efficiency and

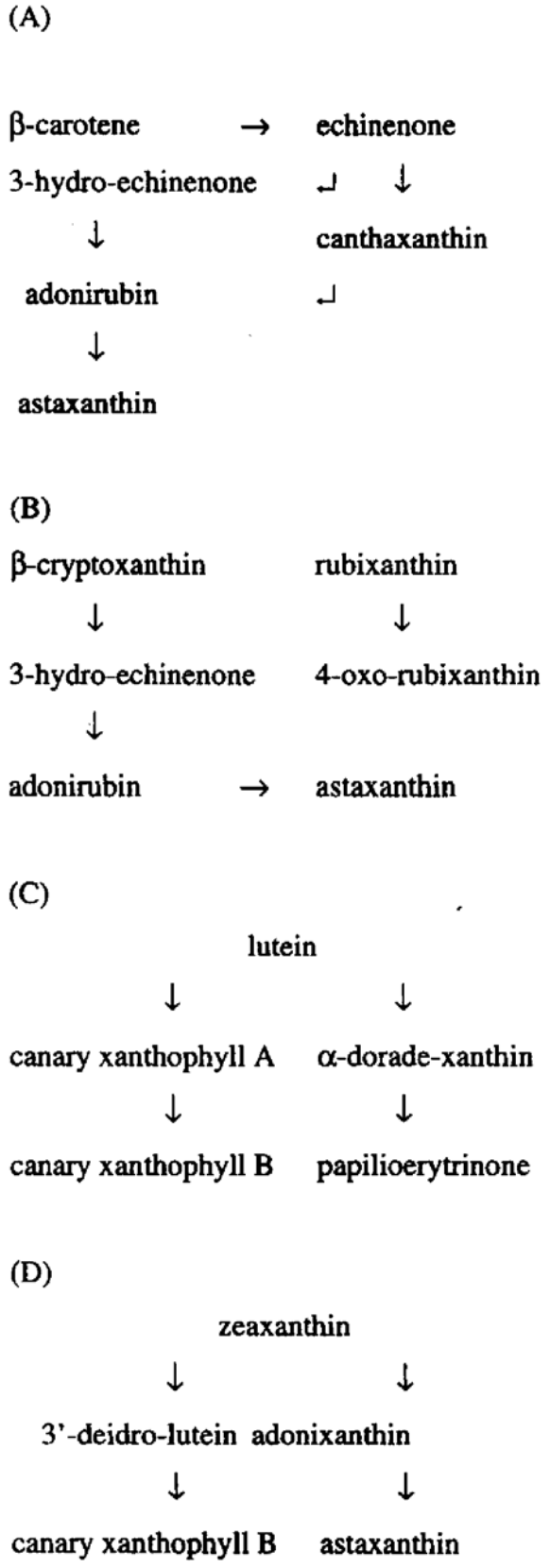

Fig. 1. Transformation of carotenoids into oxidative forms of carotenoids by birds. Adapted from Stadi (1998).

provisioning ability of males? This seems unlikely given that many males accumulate much more carotenoids than females (Hill *et al.*, 1994; Hill, 1995; Bortolotti *et al.*, 1996; Gray, 1996; Figuerola and Gutiérrez, 1998). Rather, it might be the case that males and females differ considerably in their carotenoid needs. It also seems unlikely that foraging efficiency would be the function of offspring signals based on carotenoids. Why should they signal the foraging ability of their parents? It seems much more likely that such offspring would signal their ability to metabolize, utilize and store a scarce resource. Furthermore, efficiency of carotenoid assimilation from the feed could indicate the ability of the digestive tract to assimilate other nutrients. For example aflatoxicosis in broiler chicks decreased carotenoid absorption from the intestine (Schaeffer and Hamilton, 1990). The same would also be true for other fat-soluble nutrients, and dietary supplementation with xanthophylls has been suggested as an effective way of identifying low-producing broiler breeder hens (Pinchasov *et al.*, 1992). The ubiquitous presence of carotenoids in a large number of different kinds of signals across many different classes of organisms remains puzzling. Several recent reviews have emphasized the trade-offs between competing somatic demands for carotenoids (Lozano, 1994; Olson and Owens, 1998; Saino *et al.*, 1999; von Schantz *et al.*, 1999; Blount *et al.*, 2000b). Given that carotenoids are important anti-oxidants and immunostimulants, it seems possible that their signaling function is directly reflecting such physiological properties (Lozano, 1994; Fletcher, 1992; Shykoff and Widmer, 1996; Olson and Owens, 1998; Saino *et al.*, 1999; von Schantz *et al.*, 1999).

There is considerable evidence for the physiological function of carotenoids affecting immune responses. Carotenoids are thought to play important roles in immuno-regulation and immuno-stimulation in vertebrates. β-carotene and other carotenoids enhance T and B lymphocyte proliferation, stimulate effector T cell function, enhance macrophage and cytotoxic T cell capacities, increase the population of specific types of lymphocyte, and stimulate the production of various cytokines and interleukins in humans and in other mammals (Bendich, 1989; Chew, 1993; Olson and Owens, 1998; van Poppel *et al.*, 1993; Watson *et al.*, 1991). Astaxanthin and lutein are known to enhance *in vitro* and *in vivo* antibody production to T-dependent antigens in mice, and they specifically enhance anti-sheep red blood cell antibody production in mice (Jyonouchi *et al.*, 1994, 1995). A study of cockerels fed β-carotene and canthaxanthin showed that treated birds fed carotenoids produced significantly higher antibody titers against Newcastle disease virus than control birds (McWhinney *et al.*, 1989), and experimentally increased dietary intake of β-carotene in combination with α-tocopherol (vitamin E) invoked increased immunity against *Escherichia coli* in chickens (Tengerdy *et al.*, 1990). Laying hens fed supplemental carotenoids transferred them to their chicks via the egg yolk, and produced chicks that had enhanced spleen and bursal lymphocyte function *in vitro* in comparison with chicks hatched from control hens (Haq *et al.*, 1996).

Thus carotenoids have been implicated in the enhanced proliferation of lymphocytes and cytokines, enhanced phagocytic ability of neutrophils and macrophages, and the augmentation of tumor immunity (reviews in Bendich, 1989; Chew, 1993; Edge *et al.*, 1997). Studies using human and animal cells have identified a gene, connexin 43, with a carotenoid-dependent expression that is responsible for intercellular gap junctional communication (Bertram, 1999). Thus, a number of different functions relating directly to the mechanisms and the efficient functioning of the immune system are directly affected by a number of different kinds of carotenoids.

The second major function of carotenoids that is not independent of their effects on immune function is their detoxification effects. Carotenoids and other anti-oxidants act as oxygen radical scavengers and protectors of biologically important molecules (lipids, proteins, DNA *etc.*) from the damaging potential of free radicals and products of their metabolism, acting as free radical traps and efficient quenchers of singlet oxygen (Ames, 1983; Krinsky, 1989, 1998; Edge *et al.*, 1997). Free radicals are atoms or molecules with unpaired electrons, that arise as by-products of normal metabolism and rapid cell proliferation during immune function. Free radicals attempts to pair with other electrons and thereby damage other molecules. Thus DNA, proteins and lipids, including immune molecules, can be damaged by free radicals. If free radicals are not under control, changes in membrane properties (fluidity, flexibility) and functions (intracellular signaling, enzymatic activities) arising through oxidative stress can result in immune system incompetence (Chew, 1996). Carotenoids and related anti-oxidants (including vitamin E) quench free radicals, and thereby protect lipids from oxidation, decrease immuno-

suppressive peroxides, and maintain membrane receptors that are essential for immune function. Immune cells use free radicals as a powerful weapon to kill bacteria and other infections by stimulating free radical production by macrophages (Chapple, 1997). Therefore, enhanced immune cell activity can overproduce free radicals and antioxidant protection in this case could be vital (Gille and Sigler, 1995; Jaeschke, 1995).

Production of free radicals and antioxidant defenses are usually balanced under physiological conditions, but under stressful conditions this balance can be disrupted in favor of free radical production thereby creating the situation of oxidative stress (Sies, 1997). Oxidative stress can be a result of different conditions (Halliwell, 1996) including inadequate diet-derived antioxidant availability, lack of dietary proteins and inadequate synthesis of metal-binding proteins, excess production of free radicals as a result of toxic compound metabolism and excessive activation of natural radical-producing systems such as phagocytes in inflammation.

Carotenoids are considered an important group of natural antioxidants and their antioxidant activities *in vitro* have received substantial attention in recent years (Krinsky, 1989, 1998; Edge *et al.*, 1997; Rice-Evans *et al.*, 1997; Bast *et al.*, 1998; Rao and Agarwal, 1999). Therefore carotenoids are considered to possess high antioxidant activity and perform their protective function in association with other antioxidants forming an antioxidant system of the cell (Surai, 1999).

Antioxidant activities of carotenoids *in vivo* have received less attention and are characterized in papers published mainly during the last 5 years. They are reviewed in Table 1.

Most research on antioxidant activities of carotenoids has concentrated on β-carotene since this is a widely available and an important carotenoid in human nutrition. Moreover, antioxidant protection by lutein, zeaxanthin, astaxanthin and other carotenoids have been demonstrated. Different *in vivo* systems were employed including whole body, body-derived tissues (*e.g.*, egg yolk, erythrocytes, LDL) or organelles (microsomes) as well as model systems based on cell culture. In most cases, lipid peroxidation expressed as thiobarbituric acid reactive substances (TBARS) or malondialdehyde (MDA) accumulation were used. Efficiency of carotenoids as antioxidants varied depending on the system used, but it was more pronounced under stressful conditions (*e.g.*, zinc deficiency, iron overload, UV or X-ray treatment, presence of CCl_4 or mycotoxins). All these results taken together clearly indicate a high antioxidant potential of individual carotenoids under stressful conditions, but more importantly synergistic effects of carotenoids with vitamin E (Böhm *et al.*, 1997; Palozzza and Krinsky, 1992) and between individual carotenoids (Stahl *et al.*, 1998), carotenoids and vitamin C (Böhm *et al.*, 1998), and carotenoids and antioxidant enzymes (Blakely *et al.*, 1988) have been described.

Based on this information we can suggest different strategies for male and female birds in relation to antioxidant use. The female heavily invests in its progeny by transferring antioxidants into their egg. Since the hatching process is considered as oxidative stress (Surai, 1999), the antioxidant protection is vital at this stage of development. In this respect, it is interesting to underline that carotenoid and vitamin E (two major natural antioxidants) concentrations in the embryonic tissues reach the maximal level at hatching (Surai *et al.*, 1996, 1998) providing maximal antioxidant protection to tissues with high levels of polyunsaturated fatty acids (Speake *et al.*, 1998) that are vulnerable to free radical attack (Surai, 1999). Since carotenoids are not accumulated in the body to any great extent (Surai and Speake, 1998; Surai *et al.*, 1999; Surai *et al.*, 2000), there must be a premium on a female's ability to transfer them to the egg yolk. For example, in gulls second and third eggs usually contained less carotenoids than first eggs (Royle *et al.*, 1999). Maternally-derived carotenoids in egg yolk reduce susceptibility to oxidative stress (Surai and Speake, 1998), and probably they preserve passively-acquired antibody from oxidation in embryos and chicks (Haq *et al.*, 1996; review in Blount *et al.*, 2000). Oxidative stress arising from the production of free radicals is likely to be most prominent in individuals with high activity levels, but also in rapidly growing embryos because of their high levels of oxidative metabolism (Vleck and Bucher, 1998). In species of salmonid fish, experimental supplementation of the diet with astaxanthin resulted in higher concentrations of anti-oxidants in muscle and liver, increased levels of complement, phagocytosis, lymphocyte proliferation and natural cytotoxicity (Verlhac *et al.*, 1995). Furthermore, dietary carotenoid supplementation reduced mortality after experimental challenge with *Aeromonas salmonicida* (Christiansen *et al.*, 1995), and reduced levels of oxidative stress (Nakano *et al.*, 1999). Xanthophylls possess anti-oxidant activities

Table 1. A review of the antioxidant action of carotenoids

System	Effect	Reference
Day old chicks hatched from carotenoid enriched eggs	Increased concentrations of lutein, citranaxanthin, canthaxanthin and caretenoic acid were associated with decreased TBARS accumulation in the egg yolk, yolk sac membrane and liver	Surai and Speake, 1998
Male chickens fed on the diets supplemented with either β-carotene, zeaxanthin or canthaxanthin for 36 days	Chickens given β-carotene or zeaxanthin had a reduced susceptibility to oxidant stress in liver compared with unsupplemented controls	Woodall *et al.*, 1996
Vitamin E deficient chickens	Ethyl-B-apo-8^{1}-carotenoate in feed has a protective effect	Prohaszka, 1996
Vitamin E and selenium deficient chickens	Canthaxanthin in the feed decreased lipid peroxidation in hepatic mitochondria	Mayne and Parker, 1987; 1989
Broiler chickens	β-Carotene in the chicken diet at 15 ppm acted as antioxidant in fresh and cooked meat but was prooxidant at 50 ppm	Ruiz *et al.*, 1999
Mice supplemented with β-carotene and injected with CCl_4	Urine and kidney TBARS were reduced, inverse correlation between β-carotene levels in the kidney, liver and lung and TBARS accumulation was found	Iyama *et al.*, 1996
Mice fed on the diet supplemented with β-Carotene	The red blood cell phospholipid hydroperoxides showed a significant decrease followed by an increase of β-carotene intakes	Nakagawa *et al.*, 1996
Mice with developing tumors	Lipid peroxidation in tumors was lower after feeding high dose of astaxanthin	Chew *et al.*, 1999
Female mice inoculated with tumor cells	Low levels (0.002 and 0.02%) of dietary lutein lowered lipid peroxidation	Park *et al.*, 1998
Hairless mice exposed to UV radiation	β-Carotene or palm fruit carotene in the diet decreased TBARS accumulation in the skin	Someya *et al.*, 1994
Male albino mice and rats irradiated with X-ray	β-Carotene in the diet produced significant radiation-protective effects in the animals	Kazanskaya *et al.*, 1994
Mice exposed to X-radiation	Dietary lycopene increased the survival rate	Forssberg *et al.*, 1959
Rats exposed to whole-body irradiation	The effect of irradiation was partially cured by supplementation with β-carotene, therefore providing protection *in vivo* against the cellular damage by free radicals induced after whole-body irradiation.	BenAmotz *et al.*, 1996 Umegaki *et al.*, 1994
Rats	Dietary supplementation of astaxanthin reduced the lysis of erythrocytes subjected to oxidative stress	Miki, 1991, Niki, 1991
Weanling female rats fed on diets containing 10% oxidized soybean oil	Dietary supplementation of β-Carotene reduced hepatic and erythrocyte peroxidation	Levin *et al.*, 1997
Rats fed on antioxidant-deficient diet	β-Carotene dietary supplementation significantly reduced production of TBARS during spontaneous oxidation of heart homogenates	North and Tappel, 1997.
Zinc-deficient rats	Enrichment of zinc-deficient diet with β-carotene improved erythrocyte osmotic fragility and TBARS levels	Kraus *et al.*, 1997
Rats with iron overload	Dietary β-carotene supplementation significantly reduced lipid peroxidation in the liver	Whittaker *et al.*, 1996
Male Fischer 344 rats fed on a diet supplemented with 10 ppm lycopene	An increase in serum thiols and a decrease in serum TBARS was observed in rats fed the lycopene diet.	Jain *et al.*, 1999
Weanling male rats fed on Torula yeast-based diet unsupplemented or supplemented with β-Carotene	β-Carotene supplementation had a protective effect against oxidative stress lowering TBARS accumulation and enzyme release from erythrocytes	Zamora *et al.*, 1991
Vitamin C deficient guinea pigs treated with CCl_4	Injection with β-carotene significantly decreased ethane and pentane production	Kunert and Tappel, 1983

Table 1. Continued

System	Effect	Reference
Rainbow trout fed on oxidized oils	Astaxanthin in the form of red yeast significantly decreased levels of lipid peroxides in the liver	Nakano *et al.*, 1999, Nakano *et al.*, 1995
Atlantic salmon supplemented with astaxanthin	Increased resistance to challenge with *Aeromonas salmonicida*, higher levels of antioxidant vitamins in the muscle and liver.	Christiansen *et al.*, 1995
Women on carotenoid-deficient diet	Inclusion of β-carotene in the diet normalized elevated levels of TBARS in serum	Dixon *et al.*, 1994
Elderly women	Supplementation with β-carotene increased the plasma antioxidant capacity	Meydani *et al.*, 1994
Children with cystic fibrosis	Supplementation with β-carotene normalized enhanced levels of MDA in the serum	Lepage *et al.* 1996
Children with cystic fibrosis	Supplementation with β-carotene increased resistance of LDL to oxidative stress	Winklhofer-Roob *et al.*, 1995
Cystic fibrosis patients	Oral β-carotene supplementation significantly decreased plasma MDA concentrations	Winklhofer-Roob *et al.*, 1996 Rust *et al.*,1998
Healthy human subjects	Lycopene from tomato products lowered the oxidative damage to lipids, lipoproteins and DNA	Rao and Agarwal, 1999
Patients with erythropoitic protoporphyria and similar diseases.	β-Carotene prevents or lessens photosensitivity	Mathews-Roth, 1986
Human subjects consuming tomato juice, spaghetti sauce, or tomato oleoresin	A significant increase in serum lycopene level and diminished levels of serum TBARS.	Rao and Agarwal., 1998
Healthy women consuming tomato products	After *ex vivo* treatment with hydrogen peroxide lymphocyte DNA damage decreased by 33–42%	Riso *et al.*, 1999
Young human male subjects fed on a carotenoid-free diet	β-Carotene consumption decreased serum lipid peroxide levels	Mobarhan *et al.*, 1990, Gottlieb *et al.*, 1993
Human smokers	Lipid peroxidation measured by breath-pentane output was significantly reduced by β-carotene supplementation	Allard *et al.*, 1994
Cultured rat hepatocytes treated with CCl_4	Inclusion in the medium α-carotene, β-carotene, lycopene or lutein protected cultured rat hepatocytes from CCl_4,-induced injury	Kim H. 1995
Murine normal and tumor thymocytes	β-Carotene and canthaxanthin added in the medium inhibited tret-butyl hydroperoxide-stimulated MDA formation	Palozza *et al.*, 1996
Rat kidney fibroblasts after UV-light stress	β-Carotene, lutein or astaxanthin protected against oxidative stress	O'Connor and O'Brien, 1998
Embryonic rat hippocampal cultures exposed to ethanol	β-Carotene, completely ameliorated cell loss from ethanol and increased survival by 12–18% indicating in vitro antioxidative neuroprotection against evelopmental ethanol exposure	Mitchell *et al.*, 1999
Mouse embryo fibroblast 10T1/2 cell	β-Carotene, canthaxanthin, lutein, lycopene or α-carotene inhibited lipid peroxidation	Zhang *et al.*, 1991
Hamster ovary cells with induced oxidative stress	Inclusion of β-carotene in the medium prevented formation of sister chromatid exchanges	Weitberg *et al.*, 1985
Primary cultures of chicken embryo fibroblasts treated with paraquat	Astaxanthin enrichment of the medium protected against oxidative stress	Lawlor and O'Brien, 1997
Chicken embryo fibroblasts	Incorporation of β-carotene into paraquat treated cells protected against oxidative stress	Lawlor and O'Brien, 1997
Chicken hepatocytes treated with T-2 toxin	Lutein and lycopene reduced the cytotoxic prooxidative effect of T-2 toxin	Leal *et al.*, 1998
Human liver cell line HepG2	β-Carotene or lutein in the medium partially or completely protected against oxidant-induced lipid peroxidation	Martin *et al.*, 1996
Human promyelocytic leukemia HL-60 cells	β-Carotene protected DNA breaking by peroxynitrous acid	Hiramoto *et al.*, 1999

Human monocyte-macrophages incubated for 24 h with LDL	Inclusion in the medium canthaxanthin, β-Carotene or zeaxanthine inhibited LDL oxidation	Carpenter *et al.*, 1997

Table 1. Continued

System	Effect	Reference
Human monocyte-derived macrophages in the presence of $CuSO_4$.	β-Carotene enrichment of LDL as a result of dietary supplementation significantly reduced LDL oxidation	Levy *et al.*, 1996
Cultured rat Ito cells treated with CCl_4	Zeaxanthin dipalmitate significantly decreased cellular MDA level	Kim *et al.*, 1997
In vitro cell culture	Inclusion of β-carotene in the medium decreased TBARS accumulation	Franke *et al.*, 1994
Human aortic endotelial cells in culture and LDL	Enrichment of LDL with β-carotene by dietary means markedly inhibited lipid hydroperoxide formation	Dugas *et al.*, 1999, Dugas *et al.*, 1998
LDL in cell-free or cellular system in the presence Cu^{2+}	β-Carotene inhibited the oxidative modification of LDL in both systems	Jialal *et al.*, 1991
Human LDL, rich in β-carotene and lycopene, prepared from a volunteer following supplementation with tomato juice	Accumulated cholesteryl ester hydroperoxides decreased when the suspensions containing these LDL were subjected to a single oxygen-generating system improving the antioxidant defence against the attack of single oxygen.	Oshima *et al.*, 1996
Salmonella typhimurium model system	Lutein inhibited the mutagenicity of aflatoxin B_1 in a dose-dependent manner.	DeMejia *et al.*, 1997
Red pigmented and two mutant colorless strains bacterium. *Deinococcus radiodurans*	The colorless strain was significantly more s ensitive to H_2O_2	Carbonneau*et al.*, 1989
Pigmented yeast, *Rhodotorula mucilaginosa*	Addition of β-carotene to the medium decreased oxidant-induced cytotoxicity	Moore *et al.*, 1989
Rat liver microsomes	The presence of β-carotene or astaxanthin in the membrane inhibited the loss of α-tocopherol providing antioxidant protection	Nakagawa *et al.*, 1997
Rat liver microsomes	Iron-dependent peroxidation, enhanced by adriamycin, was inhibited by β-carotene by more than 60%	Vile and Winterbourn, 1998.

comparable with those of carotenes in various *in vitro* and *in vivo* systems (Miller *et al.*, 1996; Stahl *et al.*, 1998; Rice-Evans *et al.*, 1997). Carotenoids together with other antioxidants (vitamin E) transferred to the egg or synthesized by the developing embryo (ascorbic acid, reduced glutathione or antioxidant enzymes) build an effective antioxidant system responsible for maintaining protection against damaging effects of free radicals and products of their metabolism (Surai, 1999). Thus a considerable amount of evidence implicates various kinds of carotenoids in the control and destruction of free radicals and thus as a factor limiting oxidative stress.

If carotenoids generally have positive effects on health status, we must also consider whether there are any negative effects of excess carotenoids. We are only aware of a single study showing a significantly increased frequently of lung cancer in a double-blind factorial design with crossed α-tocopherol and β-carotene treatments (Mayne, 1996). Numerous other studies of diverse organisms have provided very high levels of carotenoids in experiments, but without any clear negative effects. Although the study cited above may be important, we should not discard the possibility that the significant effect may be due to an unlikely chance event. Costs of excess carotenoids may be paid in terms of increased risks of predation if carotenoids are allocated to signals that attract predators. An experiment by Götmark and Olsson (1997) showed that great tits *Parus major*, which are naturally yellow, when dyed with a bright red color had an increased risk of predation. This suggests that there would be natural selection against mutations that transform ingested carotenoids into other substances, but also that there

would be selection against a change in choice of diet. Further studies of the potential negative effects of carotenoids are needed.

A completely different strategy in relation to natural antioxidants especially carotenoids could be suggested for males. In this case birds can afford to use more carotenoids for functions unrelated to egg production, in particular for display.

In general, carotenoid effects are not restricted to their immunostimulating or antioxidant functions, but are much wider. In particular carotenoid-modulating properties with respect to different toxic and mutagenic compounds are of interest for wild bird biology. For example β-carotene, astaxanthin and canthaxanthin protect against initiation of liver carcinogenesis by aflatoxin B1 through the change in aflatoxin metabolism towards detoxification pathways (Gradelet *et al.*, 1998; Gradelet *et al.*, 1997; He and Campbell, 1990). The inhibitory mechanism of lutein against the mutagenicity of aflatoxin B1 (de Mejia *et al.*, 1997) and 1-nitropyrene (de Mejia *et al.*, 1997) is considered to be the result of formation of complexes with these compounds or their metabolites limiting their availability. Low levels of dietary lutein in mice lowered mammary tumor incidence, tumor growth and increased tumor latency (Park *et al.*, 1998). Similarly zeaxanthin is known to provide protection against CCl_4 by antioxidant and non-antioxidant mechanisms (Kim *et al.*, 1997). It has been concluded that the protective antimutagenic action of carotenoids is associated with inhibition of the metabolic activation of the different promutagens (Rauscher *et al.*, 1998). Modulation of genotoxic and related effects by carotenoids in experimental models have been reviewed recently by De Flora *et al.* (1999).

Therefore carotenoids play important roles in protection against free radicals and different mutagenic and toxic compounds as well as activating immune system. By these means carotenoids provide a vital physiological mechanisms for successful survival and reproduction of domestic and free living birds.

2. CAROTENOID-BASED SIGNALS IN SEXUAL SELECTION AND PARENT-OFFSPRING INTERACTIONS

Here we will deal with two different kinds of signals: Sexual signals such as secondary sexual characters that are directed to other adult conspecifics of the same or the other sex and offspring signals directed towards their parents or other adults. The mechanism of signals may reveal information about their function. In particular, the morphological and physiological basis of a signal may provide information about what is being signaled, *i.e.* what have been the selective forces shaping the evolution of the signal. For example, carotenoid-based signals are likely to have functions relating to the acquisition and/or the metabolism of carotenoids. We would like to emphasize that the two kinds of signals analyzed here do not exhaustively reflect the role of carotenoids in signals. On the contrary, many warning signals in social insects such as bees, bumblebees and wasps are based on carotenoids. Similarly, floral coloration often has a carotenoid component, but to the best of our knowledge it remains undetermined whether the presence of carotenoids in these contexts has a specific function.

2.1. Frequency of carotenoid-based signals

Carotenoid-based sexual signals are common in invertebrates, fish, amphibians, reptiles, birds and mammals. A comparative study of the frequency of carotenoid-based plumage signals in North American birds revealed that carotenoid-based plumage and sexual dichromatism are positively correlated while that is not the case for melanin-based or structural colors (Gray, 1996). Although this paper contains a number of errors in terms of classification of species with respect to whether plumage traits are carotenoid-based, it seems clear that a very large fraction of sexual signals in birds are based on carotenoids. Similar analyses are absent from other taxa, so we still need quantitative information about their abundance in other vertebrates and in invertebrates.

Superficially, it may seem strange that such a large fraction of signals are based on chemical components that cannot be synthesized by the animals themselves, but have to be ingested. Why should that be the case? We will address this question in Section 3. However, first we will summarize the literature investigating whether carotenoid-based signals are preferred by receivers.

2.2. Evidence that brighter carotenoid-based signals are preferred

2.2.1. Sexual signals

Numerous studies have addressed whether current sexual selection favors the expression of extravagant

Table 2. Sexual selection in relation to carotenoid based signals

Species	Evidence	Obs/Exp	Reference
Fish			
Gasterosteus aculeatus	Brighter males have higher success	Obs	McPhail, 1969
Gasterosteus aculeatus	Brighter males have higher success	Obs	Semler, 1971
Gasterosteus aculeatus	Brighter males have higher success	Obs	Rowland, 1982
Gasterosteus aculeatus	Brighter males have higher success	Obs/Exp	Bakker and Sevenster, 1983
Gasterosteus aculeatus	Brighter males have higher succe	Exp	Milinski and Bakker, 1990
Poecilia reticulata	Brighter males have higher success	Obs	Endler, 1980
Poecilia reticulata	Brighter males have higher success	Obs	Endler, 1983
Poecilia reticulata	Brighter males have higher success	Obs	Kodric-Brown, 1985
Poecilia reticulata	Brighter males have higher success	Obs	Houde, 1987
Poecilia reticulata	Brighter males have higher success	Obs	Houde and Torio, 1992
Poecilia reticulata	Brighter males have higher success	Obs	Kodric-Brown, 1989
Birds			
Agelaius phoeniceus	Brighter males have higher succe	Exp	Peek, 1972
Agelaius phoeniceus	Brighter males have higher succe	Exp	Smith, 1972
Agelaius phoeniceus	Brighter males have higher succe	Exp	Hansen and Rohwer, 1986
Agelaius phoeniceus	Brighter males have higher succe	Exp	Roskaft and Rohwer, 1987
Agelaius phoeniceus	Brighter males have higher success	Obs	Weatherhead and Boag, 1995
Cardinalis cardinalis	Brighter males have higher success	Obs	Wolfbarger, 1999
Carduelis tristis	Brighter males have higher success	Obs/Exp	Johnson *et al.*, 1993
Carpodacus mexicanus	Brighter males have higher success	Obs/Exp	Hill, 1990
Carpodacus mexicanus	Brighter males have higher success	Obs/Exp	Hill, 1994
Carpodacus mexicanus	Brighter males have higher success	Obs	Hill *et al.*, 1994a
Carpodacus mexicanus	Brighter males have higher success	Obs	Hill *et al.*, 1999
Dendroica petechia	Brighter males have higher success	Obs	Yezerinac and Weatherhead, 1997
Emberiza citrinella	Brighter males have higher success	Obs	Sundberg and Dixon, 1996
Emberiza citrinella	Brighter males have higher succe	Exp	Sundberg, 1995a
Gallus domesticus	Brighter males have higher succe	Exp	von Schantz *et al.*, 1995
Gallus gallus	Brighter males have higher success	Obs	Zuk *et al.*, 1990a
Gallus gallus	Brighter males have higher success	Obs	Zuk *et al.*, 1990d
Gallus gallus	Brighter males have higher success	Obs	Zuk *et al.*, 1992
Gallus gallus	Brighter males have higher succe	Exp	Ligon *et al.*, 1990
Gallus gallus	Brighter males have higher succe	Exp	Ligon *et al.*, 1996
Gallus gallus	Brighter males have higher success	Obs	Johnsen and Zuk, 1996
Gallus gallus	Brighter males have higher succe	Exp	Zuk *et al.*, 1990c
Geothlypis trichas	Brighter males have higher succe	Exp	Lewis, 1972
Hirundo rustica	Brighter males have higher success	Obs	P. Ninni *et al.*, unpublished manuscript
Lagopus lagopus	Brighter males have higher success	Obs/Exp	Hannon and Eason, 1995
Lagopus mutus	Brighter males have higher success	Obs	Brodsky, 1988
Lagopus mutus	Brighter males have higher success	Obs	Holder and Montgomerie, 1993
Meleagris gallopavo	Brighter males have higher success	Obs/Exp	Buchholz, 1995
Phasianus colchicus	Brighter males have higher succe	Exp	Hillgarth, 1990
Phasianus colchicus	Brighter males have higher succe	Exp	Mateos and Carranza, 1995
Ploceus cucullatus	Brighter males have higher succe	Exp	Collias *et al.*, 1979
Taeniopygia birchenovi	Brighter males have higher succe	Exp	Burley, 1986a
Taeniopygia guttata	Brighter males have higher succe	Exp	Burley, 1986a
Taeniopygia guttata	Brighter males have higher succe	Exp	Burley and Coopersmith, 1987
Taeniopygia guttata	Brighter males have higher succe	Exp	Burley, 1988
Taeniopygia guttata	Brighter males have higher success	Obs	Burley and Price, 1991
Taeniopygia guttata	Brighter males have higher success	Obs/Exp	Burley *et al.*, 1994
Taeniopygia guttata	Brighter males have higher succe	Exp	Collins *et al.*, 1994
Taeniopygia guttata	Brighter males have higher succe	Exp	Burley *et al.*, 1996
Taeniopygia guttata	Brighter males have higher succe	Exp	Burley, 1996
Taeniopygia guttata	Brighter males have higher succe	Exp	Bennett *et al.*, 1996
Xanthocephalus xanthocephalus	Brighter males have higher succe	Exp	Rohwer and Røskaft, 1989

signals (see Andersson, 1994). Carotenoid-based signals comprise a large fraction of these studies. A summary of the findings of such studies is provided in Table 2. Generally, males with more brightly colored signals are preferred as mates, or win fights with other males. For example, studies of zebra finches *Taeniopygia guttata* have shown that females prefer males with bright red or orange leg bands, while they behave indifferently to males with green bands (Burley, 1986a, 1986b). Experiments with coloration of signals such as combs of pheasants *Phasianus colchicus* and jungle fowl *Gallus gallus* show that females have clear preferences for males with brighter coloration (Hillgarth, 1990; Zuk *et al.*, 1990a, 1990c, 1990d; Mateos and Carranza, 1995). Similarly, the red color of the belly of male three-spined sticklebacks *Gasterosteus aculeatus* is preferred by females (Bakker and Sevenster, 1983; Milinski and Bakker 1990). Bright feather color of male village sparrows *Ploceus cucullatus,* yellowhammers *Emberiza citrinella* and house finches *Carpodacus mexicanus* is preferred by females during mate choice (Collias *et al.*, 1979; Hill, 1990; Sundberg, 1995).

Thus there is overwhelming evidence for males with more bright red or yellow color having a mating advantage compared to less colorful competitors. However, we do not know whether the intensity or the persistence of female preferences for carotenoid-based signals differ from that of signals with other biochemical bases.

2.2.2. *Offspring signals*

Offspring signals such as bright gapes and extravagant colors may provide parents with information about the current state and the future survival prospects of offspring (Kilner and Johnstone, 1997; Saino *et al.*, 2000). Theoretical studies have suggested that exaggerated begging signals may evolve to reflect the need of offspring (Parker and Mock, 1987; Parker *et al.*, 1989; Godfray, 1991, 1995). Even the health status of individual offspring may be revealed reliably from their begging signals when the functional basis for the signal also reflects the physiological components involved in coping with the rigors of life (Saino *et al.*, 2000). Since carotenoids are used for free radical scavenging, and also for modulation and enhancement of immune function (see Introduction), we can imagine a trade-off between allocation to signals and other functions.

Several studies have investigated whether parents respond to such exaggerated carotenoid-based signals of dependent offspring (Table 3). Offspring of several species of coots and moorhens have brightly yellow and red colored head feathers that are completely absent in adults. The mere presence of these brightly colored feather traits suggests that they have a function, since predators are likely to cue in on these exaggerated structures. Lyon *et al.* (1994) manipulated the length of the head feathers of American coots *Fulica americana* by cutting these from some offspring. There was a dramatic reduction in performance of these offspring due to parental neglect. Apparently, this signal has evolved as a means of offspring communicating with their parents, although the study did not explain why such signals are not ubiquitous.

Kilner (1997) and Götmark and Ahlström (1997) showed independently that nestlings of canaries *Serinus canaria* and blue tits *Parus caeruleus* with experimentally more brightly colored gapes received more food than their nest mates. Since nestlings of both species have considerably more brightly colored gapes than adults, these signals seem exaggerated relative to what one should expect from the appearance of adults. The exact basis of the gape color was not revealed, although Kilner (1997) suggested for the canary that this was related to blood flow through the mouth tissue, producing sudden surges in coloration. A subsequent study by Saino *et al.* (2000) showed that a challenge of

Table 3. Parental preference for offspring in relation to their signals

Species	Evidence	Obs/Exp	Reference
Fulica americana	Parents allocate more food to offspring with larger red feathers	Exp	Lyon *et al.*, 1994
Hirundo rustica	Parents allocate more food to offspring with more bright gape	Exp	Saino *et al.*, 2000
Parus caeruleus	Parents allocate more food to offspring with more bright gape	Exp	Götmark and Ahlström 1997
Serinus canaria	Parents allocate more food to offspring with more bright gape	Exp	Kilner 1997

the immune system of nestling barn swallows *Hirundo rustica* with sheep red blood cells reduced gape color compared to that of nestlings receiving a control injection. Interestingly, the gape color could be restored to its previous level by provisioning of nestlings with lutein, which is the most common carotenoid circulating in the blood in nestling barn swallows. In other words, the signal tracked the current state of the nestlings, and it could be altered reversibly by simple administration of extra carotenoids.

There is some evidence of offspring signals being carotenoid-based although we know very little about the functional significance of such signals. Only a single study has revealed a direct link between gape color and a function in terms of an indicator of immunocompetence.

3. WHAT DO CAROTENOID-BASED SIGNALS REVEAL?

The function of signals can be inferred from the design of signals and how they respond to manipulations of environmental conditions. In the following, we will address the question to which extent carotenoid-based signals may reveal: (1) foraging efficiency, (2) disease status, (3) immunocompetence, and/or (4) anti-oxidant potential. These functions are not necessarily independent, but may act in concert.

3.1. Foraging efficiency and carotenoid-based signals

Since carotenoids have to be ingested by most organisms in order to be used for physiological or signaling functions, it is straight forward to suggest that their level of display in signals reflects foraging efficiency (Brush and Power, 1976; Endler, 1980, 1983; Kodric-Brown, 1985; Hill, 1992; Hill *et al.*, 1994). In particular, animals living in carotenoid-poor environments may produce secondary sexual characters with a low intensity of coloration (Brush and Power, 1975; Endler, 1980, 1983; Slagsvold and Lifjeld, 1985; Hill, 1993). This is particularly evident among animals raised in captivity (Brush and Power, 1975; Goodwin, 1984; Scheidt, 1989; Putnam, 1992; Hill, 1993). For example, provisioning of food with a high carotenoid content to molting birds would mimic the situation by which certain individuals were able to locate and ingest food with a high carotenoid content. A field study by Grether *et al.* (1999) suggested that guppies *Poecilia reticulata* ingested food items with a higher carotenoid content when living in a carotenoid-rich environment, and this affected their subsequent coloration. However, even if it could be demonstrated that individuals have evolved food choice specifically designed to maximize carotenoid intake, this would still beg the question why this food choice had been perfected during evolution in the first place.

We suggest that the diverse occurrence of carotenoid-based signals among animals is unlikely to be explained solely by an evolutionary history of selection for individuals with superior foraging efficiency. For example, such a hypothesis cannot explain the evolution of carotenoid-based signals in offspring that depend on their parents for food (see Introduction). Carotenoid-based signal expression is bound to be affected by foraging efficiency, but we suggest that the underlying function of these signals cannot be deduced unless we consider the various competing uses of carotenoids. In other words, the function probably cannot be revealed without considering the physiological processes that implicate carotenoids in anti-oxidant activity and immune function.

3.2. Limitation of carotenoids

The entire argument about signals being reliable depends upon the signal being costly. Costs may reflect those acquired during production and/or maintenance. Production costs of carotenoid-based signals rely heavily on carotenoids being a limiting resource, since the absence of a trade-off between allocation to signals and physiological processes would allow most individuals to produce exaggerated signals without being penalized. The question of carotenoid availability has two components: (1) Is carotenoid availability limiting the expression of signals? (2) To which extent does availability and turnover affect the expression of signals?

A number of different studies have investigated to which extent signals can become more exaggerated by provisioning of extra carotenoids (Table 4). Two studies of guppies have demonstrated that provisioning with extra carotenoids increases the brightness of orange color spots of males. Kodric-Brown (1985, 1989) in a series of laboratory experiments revealed that guppy males fed extra carotenoids became more brightly colored. She thus inferred that diet choice would affect male nuptial coloration. Since laboratory animals may differ from field animals in a number of different ways, it is not easy to extrapolate laboratory studies to field situations, in particular because avail-

Table 4. Carotenoid limitation of expression of carotenoid-based signals

Species	Evidence	Obs/Exp	Field/Lab	Reference
Carpodacus mexicanus	Carotenoid-rich food increases plumage color	Exp	Lab	Hill, 1992
Hirundo rustica	Carotenoids increase gape color	Exp	Field	Saino *et al.*, 2000
Larus fuscus	Carotenoid-rich food increases color of bill, legs, gape and eye ring	Exp	Field	J. D. Blount *et al.*, unpublished results
Poecilia reticulata	Carotenoid-rich food increases color of skin spots	Exp	Lab	Kodric-Brown, 1989
Poecilia reticulata	Carotenoid-rich food increases color of skin spots	Obs	Field	Grether *et al.*, 1999

ability and need may both change among environments. Grether *et al.* (1999) found in the field that carotenoid availability differed considerably among guppy populations, and most importantly that it covaried with coloration of fish collected in the field. In other words, guppies had signals that were limited in expression by lack of sufficient amounts of carotenoids.

Similar studies of three birds have shown equally clear evidence of carotenoid limitation of signals (Table 4). Captive house finch males provided with a carotenoid rich diet became more brightly red than controls when provisioned before and during molt (Hill, 1992). Gape color of nestling barn swallows in the field increased in brightness when nestlings were provided with additional carotenoids rather than a control substance (Saino *et al.*, 2000). Similarly, adult lesser black-backed gulls *Larus fuscus* provided with a carotenoid-rich diet had more brightly colored secondary sexual signals (bill, leg, gape, eye ring) than control birds not receiving carotenoid supplementation (J. D. Blount *et al.*, unpublished data).

The second question concerns the relative role of availability and turnover in determining coloration of signals. Even if carotenoids are not limiting, a high level of turnover may render carotenoids scarce during periods of extensive use. Hill (1999) suggested that markedly different levels of carotenoids in mammals, birds and fish could be taken as evidence for it being unlikely that carotenoids are severely limiting in species with high levels. However, this suggestion hinges upon the question of the rate of turnover. For example, chickens experimentally infected with Coccidia species may lose up to 80% of their body carotenoids during a short period (Allen, 1997). Such high levels of turnover may render carotenoids relatively scarce because only the most carotenoid rich diet would allow an individual to replenish its resources in a short time period. Thus, we suggest that carotenoids may be limiting, not only because of limited availability in the environment, but also because their turnover may reach very high levels due to use for immune function and/or free radical scavenging.

There is limited, but convincing experimental evidence of carotenoid limitation of sexual and offspring signals under laboratory and field conditions. Further studies of different species may reveal whether this conclusion can be considered general. The relative role of turnover and availability in limiting carotenoid demands remains to be determined.

3.3. Disease status and carotenoid-based signals

Sexual signals have been hypothesized to reveal the health status of the signaler because only resistant individuals in prime condition will be able to develop the most exaggerated signals (Hamilton and Zuk, 1982). The expression of carotenoid-based signals often appears to be negatively related to parasite abundance (Table 5). As already stated, body levels of carotenoids can be reduced up to 80% during infections with Coccidia species in chickens (Allen, 1997), apparently due to free-radicals produced during the immune response (Allen, 1997). Similar findings have been reported in other studies of parasites of poultry (Bletner *et al.*, 1966; Kowalski and Reid, 1970; Yvore and Mainguy, 1972; Ruff *et al.*, 1974). The expression of secondary sexual characters was negatively affected by endoparasites of three-spined sticklebacks and guppies (Milinski and Bakker, 1990; Kennedy *et al.*, 1987; Houde and Torio, 1992). Similarly, studies of the western fence lizard *Sceloporus occidentalis* (Schall and Dearing, 1987) have revealed that more brightly colored individuals

Table 5. Reduced signal brightness for carotenoid-based signals due to parasitism

Species	Evidence	Reference
Fish		
Gasterosteus aculeatus	Males with brighter skin color have more endoparasites	Brønseth and Folstad, 1997
Gasterosteus aculeatus	Males with brighter skin color have more endoparasites	Folstad *et al.*, 1994
Gasterosteus aculeatus	Males with brighter skin color have more endoparasites	Milinski and Bakker, 1990
Gasterosteus aculeatus	Males with brighter skin color have more endoparasites	Fitzgerald *et al.*, 1993
Poecilia reticulata	Males with more bright color have fewer ectoparasites	Houde and Torio, 1992
Poecilia reticulata	Males with more bright color have fewer ectoparasites	Kinnedy *et al.*, 1987
Salvelinus alpinus	Males with more bright color have fewer endoparasites	Skarstein and Folstad, 1996
Reptiles		
Cnemidophorus arubensis	Males with more bright color have more blood parasites	Schall, 1986
Sceloporus occidentalis	Males with more bright color have fewer blood parasites	Schall and Dearing, 1987
Birds		
Agelaius phoeniceus	Males with brighter plumage have more endoparasites	Weatherhead *et al.*, 1993
Agelaius phoeniceus	Males with brighter plumage have fewer ectoparasites	Weatherhead *et al.*, 1993
Agelaius phoeniceus	Males with brighter plumage have more blood parasites	Weatherhead *et al.*, 1993
Agelaius phoeniceus	Males with brighter plumage have more ectoparasites	Weatherhead *et al.*, 1993
Agelaius phoeniceus	Males with brighter plumage have more parasites	Weatherhead *et al.*, 1993
Carduelis flammea	Males with brighter plumage have more blood parasites	Seutin, 1994
Carpodacus mexicanus	Males with brighter plumage have fewer ectoparasites	Thompson *et al.*, 1997
Carpodacus mexicanus	Males with brighter plumage have fewer endoparasites	Thompson *et al.*, 1997
Carpodacus mexicanus	Males with brighter plumage have fewer parasites	Nolan *et al.*, 1998
Carpodacus mexicanus	Males with brighter plumage have fewer parasites	Hill and Brawner, 1998
Emberiza cirlus	Males with brighter plumage have fewer blood parasites	Figuerola *et al.*, 1999
Emberiza citrinella	Males with brighter plumage have fewer blood parasites	Sundberg, 1995b
Falco sparverius	Males with brighter coloration have fewer endoparasites	Henderson *et al.*, 1995
Falco sparverius	Males with brighter coloration have fewer blood parasites	Wiehn *et al.*, 1997
Falco tinnunculus	Males with brighter plumage have more blood parasites	Korpimäki *et al.*, 1995
Gallus gallus	Males with brighter combs have fewer endoparasites	Zuk *et al.*, 1990b,d
Meleagris gallopavo	Males with brighter skin color have fewer ectoparasites	Buchholz, 1995
Meleagris gallopavo	Males with brighter skin color have fewer endoparasites	Buchholz, 1995
Meleagris gallopavo	Males with brighter skin color have fewer blood parasites	Buchholz, 1995
Parus major	Males with brighter plumage have more blood parasites	Dufva and Allander, 1995
Parus major	Young males with brighter plumage have fewer blood parasites	Hõrak *et al.*, 2000a
Parus major	Old males with brighter plumage have more blood parasites	Hõrak *et al.*, 2000a
Taeniopygia guttata	Males with brighter beaks have more ectoparasites	Burley *et al.*, 1991

had lower levels of malarial infections than pale individuals. Studies of birds have demonstrated that red plumage is more bright in male house finches with fewer parasites (Thompson *et al.*, 1997; Hill and Brawner, 1998; Nolan *et al.*, 1998). Similar findings have been reported for other species (Table 5). However, it is important to note that many studies also have reported positive relationships between intensity of coloration and parasite loads, even for malarial parasites (Table 5).

Numerous studies of parasite loads in relation to expression of secondary sexual characters have been published (review in Møller *et al.*, 1999). Although there is a slightly, but highly significant negative relationship between parasite load and signal expression (Møller *et al.*, 1999), this correlation only accounts for about 1% of the variance. It is not surprising that the evidence is not more clear cut, since many studies only have studied benign parasites with weak or no effect on the fitness of their hosts. Furthermore, since the intensity of infections with different kinds of parasites of a sample of hosts generally is not positively correlated, the abundance of parasites in general cannot be negatively correlated with the expression of signals (Møller *et al.*, 1999). This does not invalidate the original hypothesis of Hamilton and Zuk (1982), but instead emphasizes that it is more important to consider resistance rather than parasite loads.

Why should the carotenoid content of signals decrease in response to parasitism more than say melanin content or body condition? For example, Hill and Brawner (1998) have shown that only carotenoids,

not melanin, are associated with survival from an infectious disease in house finches. We see no explicit reason why there should be a specifically high turnover of carotenoids in connection with parasitism unless carotenoids played a direct role in dealing with parasitism. Thus, we suggest that the specific differential change in carotenoid contents, but not the contents of other pigments, associated with parasitism reflects their differential use, and hence their function. In fact, parasitism and infection activate the immune system, which produces free radicals to kill bacteria, and antioxidants including carotenoids are extremely important to prevent damage to immune organs and other tissues.

In conclusion, there is some evidence of parasites reducing the coloration of sexual signals of their hosts, although this is far from a ubiquitous finding (see also Møller *et al.*, 1999). Many parasites are apparently benign and should thus have little or no effect on the expression of costly signals.

3.4. Immunocompetence and carotenoid-based signals

If signals reveal the health status of the signaler because such individuals are resistant to parasites, we should expect individuals with the most exaggerated secondary sexual characters to have more efficient immune systems. This prediction has been investigated in a number of different studies (Table 6). Most studies showed that males with large secondary sexual characters were in better health status and/or were able to

Table 6. Immune function in relation to brightness of carotenoid-based signals

Species	Evidence	Reference
Fish		
Salvelinus alpinus	Males with brighter skin color have smaller leukocyte counts	Skarstein and Folstad, 1996
Salvelinus alpinus	Males with higher lymphocyte counts have more saturated color and smaller hue values	Liljedal *et al.*, 1999
Reptiles		
Psammodromus algirus	Males with brighter skin color have stronger responses to phytohaemagglutinin challenge	G. Sorci *et al.*, unpublished manuscript
Birds		
Carduelis cannabina	Males with brighter plumage have smaller spleens	Møller *et al.*, 1998
Dendrocopus major	Males with brighter plumage have smaller spleens	Møller *et al.*, 1998
Emberiza cirlus	Males with brighter plumage have higher leukocyte counts	Figuerla *et al.*, 1999
Gallus gallus	Males with brighter combs have stronger immune responses	Zuk *et al.*, 1995
Gallus gallus	Lines selected for larger combs have weaker immune systems	Verhulst *et al.*, 1999
Gallus gallus	Males with brighter colour have cell-mediated immunity, but lower levels of lymphocytes before and higher levels during the breeding season	Zuk and Johnsen, 1998
Hirundo rustica	Males with more bright plumage have less immunoglobulins	Saino *et al.*, 1999
Hirundo rustica	Offspring challenged with sheep red blood cells have less bright gapes	Saino *et al.*, 2000
Parus major	Males with more bright plumage have more heterophils	Dufva and Allander, 1995
Sylvia communis	Males with more bright plumage have smaller spleens	Møller *et al.*, 1998
Turdus merula	Males with more bright beaks have smaller spleens	Møller *et al.*, 1998
Turdus merula	Males with more bright beaks have weaker responses to sheep red blood cells, but stronger responses to phytohaemagglutinin	Preault, 1999

raise a stronger immune response to a challenge than males with smaller signals. This is consistent with the general pattern found for secondary sexual characters (review in Møller *et al.*, 1999).

Several studies demonstrated that individuals with larger secondary sexual characters were better able to raise an immune response to a challenge. For example, male European blackbirds *Turdus merula* with a more brightly colored beak produced a stronger immune response to a challenge with the lectin phytohaemagglutinin (Preault, 1999). The direct implication of carotenoids in immune function was investigated in nestling barn swallows (Saino *et al.*, 2000). Nestlings with more brightly colored gapes received more food from their parents than nestlings with pale gapes, as demonstrated by a gape coloration experiment. When the immune system was challenged by an injection with sheep red blood cells, this produced a reduction in the brightness of gape color, which could subsequently be restored by supplementation with the carotenoid lutein (the most common carotenoid in this species). Thus brightness of the signal was reversibly affected by current levels of carotenoids. A number of other studies only investigated the amount of circulating leukocytes and immunoglobulins, which should reflect the current infection status of the individual. These studies generally showed that males with more bright sexual signals had smaller concentrations of leukocytes and immunoglobulins, consistent with the prediction that males with more exaggerated signals had better current health. This was, for example, the case in adult male barn swallows in which more brightly colored males had lower concentrations of leukocytes and immunoglobulins (Saino *et al.*, 1999). Finally, a single study demonstrated that males of several different species of birds with large, carotenoid-based secondary sexual characters tended to have smaller spleens than the average male (Møller *et al.*, 1998). Since the spleen is the main storage organ for lymphocytes (Rose, 1981), this observation suggests that males with more bright plumage coloration had a smaller store of lymphocytes. This finding is consistent with the prediction than males with the brightest coloration have a better health status than males with less exaggerated sexual signals.

Carotenoids are utilized for the production and maintenance of immune system components (see Introduction). However, the mounting of a humoral immune response also directly consumes antioxidants such as carotenoids, because of the need to scavenge highly reactive oxidative metabolites arising through immune system activity. A humoral immune response causes the production of reactive oxygen species produced by the effector cells of the cell-mediated part of the immune system (Allen, 1997). This may subsequently give rise to lipid peroxidation and hence reduce the availability of carotenoids for other functions, including signaling.

In conclusion, there is considerable evidence in support of the prediction that carotenoid-based signals reflect the health status of males, and the ability of individuals to raise an immune response to a challenge with a novel antigen. Only a single study has examined the direct interaction between challenge of the immune system, carotenoid availability and the expression of the signal. More studies of this kind would be needed to determine whether carotenoid-based signals reliably reflect immunocompetence.

3.5. Detoxification potential and carotenoid-based signals

If signals reveal the potential to neutralize free radicals and thus cope with oxidative stress, we should expect that individuals with more bright signals had a greater anti-oxidative potential than the average individual in the population (von Schantz *et al.*, 1999). Only indirect evidence is available to test this prediction.

Eeva *et al.* (1998) showed that great tits *Parus major* reared closer to a source of heavy metal pollution had less intense yellow color than conspecifics from further away. It has been known for a long time that great tits from cities have more pale coloration than tits from forests (Slagsvold and Lifjeld, 1985; Hõrak *et al.*, 2000a,b). This difference has usually been attributed to lower availability of carotenoids in the urban environment. However, an alternative interpretation is that great tits from cities use a larger amount of carotenoids for free radical scavenging, since pollution is a stress factor stimulating free radical production, thus leaving a smaller amount for plumage coloration. In the study by Eeva *et al.* (1998) it was noted that the coloration of nestling great tits correlated positively with the density of green herbivorous larvae in the territory of their parents. However, since the use of carotenoids for free radical scavenging was not assessed, we cannot exclude the possibility that more carotenoids were used in areas with more heavy pollution.

A study of red coloration of adult barn swallows near the radioactively contaminated zone in Chernobyl and

in two control areas revealed weaker coloration in the contaminated area (Camplani *et al.*, 1999). Furthermore, while the intensity of red coloration was positively correlated with the expression of another secondary sexual character (the length of the outermost tail feathers) in an uncontaminated control area in Ukraine and an uncontaminated control area in Denmark, this correlation disappeared in the Chernobyl area (Camplani *et al.*, 1999). Again, a possible interpretation of these data is that male barn swallows with long tails were in need of more carotenoids for free radical scavenging in the Chernobyl area, leaving smaller amounts for plumage coloration. It is well known that radiation is a stress factor stimulating free radical production, increasing the need for anti-oxidant protection. The alternative suggestion that carotenoids simply were less available does not seem to apply, since short-tailed males and females did not have less carotenoids in their plumage. Furthermore, the annual molt takes place in the African winter quarters during December-February at a time when availability of carotenoids is unaffected by conditions in the breeding sites.

Patterns of sexual coloration in relation to known sources of free radicals are consistent with the hypothesis that more carotenoids are used for anti-oxidant activity and fewer are available for signals in the presence of large amounts of free radicals. Direct measurements of carotenoids in individuals differing in levels of free radicals are needed. Furthermore, we need information on the antioxidant potential of individuals differing in the expression of their secondary sexual characters. The prediction would be that secondary sexual characters reliably reflect the ability to cope with free radicals, with the coloration of secondary sexual characters reflecting the ability to neutralize free radicals.

4. DISCUSSION

We have reviewed the literature on the antioxidant properties of carotenoids and the prevalence of carotenoid-based signals and their use in sexual and parent-offspring signaling contexts. There is good evidence for widespread carotenoid-based signals to be strongly preferred by females, and to some extent by parents, although the number of studies addressing offspring signals is still very small. There is also some evidence for carotenoid-based signals reflecting infections with parasites, although the evidence for carotenoid-based signals revealing immunocompetence and anti-oxidant potential seems to be stronger. Although the direct functional basis for carotenoid-based signals may be either as a revealing indicator of foraging efficiency, parasite infection level or an indicator of anti-oxidant potential, the evidence presented here supports the conclusion that carotenoids are more than general indicators of foraging ability. We conclude that carotenoid-based signals may have an important role in immune function, antioxidant activity, detoxification, and as antimutagenic biochemicals, and that it is this physiological property that is the most likely universal functional basis for the reliability of such signals.

These aspects of signaling potentially have a number of implications for animal breeding and conservation. If it is the case that animal signals often are health indicators, this might have implications for the performance of individuals in their particular environment. It is possible that the use of such individuals with the most exaggerated signals in breeding programs, both in animal breeding and in conservation projects involving captive breeding programs, will facilitate successful propagation. It also seems likely that captive individuals often may suffer from carotenoid limitation, and this may have consequences for immune function and detoxification, but also for proper expression of normal signals and behavior. Many conservation programs and in particular captive breeding programs suffer from a lack of success despite captive individuals having *ad libitum* access to food, an absence of predation and excellent veterinary care. We can imagine that some of this lack of success can be attributed to the poor functioning of the immune system and the detoxification system due to lack of carotenoids. Furthermore, poor performance may arise from the fact that sexual and offspring signals are not properly developed because of a scarcity of carotenoids in the captive breeding environment. If these predictions turn out to be supported by empirical tests, these problems could readily be ameliorated by a change of diet.

5. CONCLUSIONS

The main conclusion of this review is that carotenoids have important physiological functions in free-living animals. Since a large number of sexual and other signals are based on carotenoids, and since many signals are expressed at levels well below their

maximum level of brightness, it seems likely that such signals are reflecting the immunocompetence and the antioxidant and detoxification potential of their bearers.

We would like to end by emphasizing a number of scientific questions that need considerably more attention. Most of the studies of carotenoid-based signals are based on sexual signals. Hence, we need more studies of signaling contexts other than that of sexual signaling. Although we have a certain amount of information about the role of carotenoids in the immune system and the detoxification system from carefully conducted laboratory experiments, we need more studies of the role of carotenoids in immune function and free radical scavenging, in particular under natural conditions. Furthermore, it would be important to investigate to which extent assessment of carotenoid-based signals provides reliable information about the immunocompetence and/or the anti-oxidative potential of signalers. We would like to emphasize that it is crucial to investigate to which extent signals and the immune function and detoxification properties signaled by such displays reliably predict fitness components such as fecundity and survival among parents and their descendants. Finally, we also need information about the maternal and genetic contributions to carotenoid-based signals, carotenoid-mediated immune function and free radical scavenging, since females of a number of species provide significant amounts of carotenoids to their offspring via eggs. Studies under natural conditions would be particularly revealing because both availability and use of carotenoids in nature may differ dramatically from the patterns observed in captivity. The study of interactions between carotenoids and other antioxidants (*e.g.*, vitamin E, ascorbic acid) in the embryonic development of wild birds will also provide important insights about the role of antioxidant systems in avian reproduction. For example, the question why β-carotene is not accumulated in domestic chickens, but comprises up to 30% of total carotenoids in the egg yolk of lesser black-backed gulls and some other species (P. F. Surai *et al.*, unpublished data) awaits investigation.

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