



REVIEWS

Life history trade-offs are influenced by the diversity, availability and interactions of dietary antioxidants

CARLO CATONI*†, ANNE PETERS† & H. MARTIN SCHAEFER*

*Faculty of Biology, Department of Evolutionary Biology and Animal Ecology, University of Freiburg

†Behavioural Ecology of Sexual Signals Group, Max Planck Institute for Ornithology

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The expression of most life history traits, such as immunity, growth and the development of sexual signals, is negatively affected by high levels of oxidative stress. Dietary antioxidants can reduce oxidative stress and have therefore been the focus of numerous studies in behavioural and evolutionary ecology in the last few decades. Most of this research has focused on carotenoids, neglecting a number of more common, more potent, and thereby potentially more important, antioxidants, such as polyphenolic antioxidants. However, the effects of several classes of antioxidants on different life history traits have been thoroughly investigated in medical and animal-breeding studies. We suggest that behavioural and evolutionary studies will benefit from incorporating these advances. By reviewing the literature on the effects of antioxidants on life history traits in fish, birds and mammals, we develop a broad framework for dietary antioxidants. Fundamental properties of antioxidants, in particular their biochemistry, their potency and the interactions between them affect their relative relevance for life history traits. Based on tissue affinity, we distinguish between two categories of dietary antioxidants: focal antioxidants that are intrinsically important for a given trait and nonfocal antioxidants that influence traits only indirectly. Furthermore, we show how temporal and spatial environmental variability in antioxidant availability, as well as individual variation in food selection, may generate interindividual differences in the expression of life history traits. Finally, we suggest future research lines and experimental designs that may provide basic information needed to advance our knowledge of the ecological and evolutionary relevance of dietary antioxidants.

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Many physiological functions depend on a balance between reactive species (e.g. reactive oxygen and nitrogen compounds) and antioxidant defences (Halliwell & Gutteridge 2006). Reactive species are metabolic by-products

essential for energy supply, chemical signalling and detoxification (Halliwell & Gutteridge 2006). However, overexposure to reactive species leads to oxidative stress causing cell and tissue damage and, finally, premature ageing and eventually degenerative diseases (Finkel & Holbrook 2000). Oxidative stress is reduced by a complex network of antioxidants, among which dietary antioxidants play a fundamental role (reviewed in Vertuani et al. 2004). In the last two decades, a wealth of studies in medicine and animal health has demonstrated that dietary antioxidants may increase immunocompetence (Hughes 1999a), slow down the process of ageing (Ames

Correspondence: C. Catoni, Faculty of Biology, Department of Evolutionary Biology and Animal Ecology, University of Freiburg, Biology 1, Hauptstrasse 1, 79104 Freiburg, Germany (email: carlo.catoni@uranus.uni-freiburg.de). A. Peters is at the Behavioural Ecology of Sexual Signals Group, Max Planck Institute for Ornithology, Vogelwarte Radolfzell, 78315 Radolfzell, Germany.

et al. 1993), and reduce the occurrence of chronic diseases such as cancer or Alzheimer's (Brash & Havre 2002).

These effects make dietary antioxidants ideal candidates to influence key traits in life history (von Schantz et al. 1999). The same may be true for endogenous antioxidants, such as superoxide dismutase or uric acid (Vertuani et al. 2004). However, little is known about their interindividual variation and their relevance for behavioural traits. We thus focus our review exclusively on dietary antioxidants.

Dietary antioxidants comprise a group of several hundred different compounds. Although medical studies have investigated the effects of several antioxidants on various traits, behavioural ecologists have, with a few recent exceptions (e.g. de Ayala et al. 2006; Hōrak et al. 2007; Pike et al. 2007b; Catoni et al., in press), mostly focused on a single class of antioxidants, carotenoids. In the last two decades, carotenoid-based signalling has become a model system in sexual selection theory to analyse the role of oxidative stress in life history trade-offs such as those between self-maintenance and reproduction (McGraw & Ardia 2004). However, the relevance of carotenoids as antioxidants might have been overestimated, at least in birds (Hartley & Kennedy 2004; Costantini & Møller 2008). Consequently, although the focus on carotenoids has generated many valuable insights into animal physiology and the evolution of animal signals, it has, at the same time, limited our understanding of the general relevance of antioxidants in evolutionary ecology and physiology.

In this review, we aim to rejoin the progresses in medical and veterinary science with those from behavioural ecology and thereby develop an encompassing framework for the ecological and evolutionary significance of dietary antioxidants. To this end, we first consider the basic characteristics of the most common antioxidant classes, their bioavailability, their tissue distribution and their interactions. Based upon this

information, we discuss the relevance of single antioxidants for various life history traits of mammals, birds and fish. Furthermore, we show how behaviourally and environmentally induced variations in antioxidant intake may affect the expression of life history traits. Finally, we identify critical gaps in our current knowledge and briefly outline methods and experiments to address them.

BASIC CHARACTERISTICS OF DIETARY ANTIOXIDANTS

Dietary antioxidants are mainly secondary metabolites that plants synthesize to protect themselves against oxidative stress. According to their chemistry, they may be grouped into four classes: vitamin C (ascorbic acid); vitamin E (tocopherols); carotenoids (e.g. α - and β -carotenes, lycopene, lutein); and polyphenolic antioxidants. The latter are a very diverse group among which phenolic acids and flavonoids such as anthocyanins and quercetin are the most important antioxidants. The four classes of dietary antioxidants differ dramatically in their mean antioxidant potency, absorbability and environmental availability. Flavonoids and phenolic acids are the antioxidants with the highest environmental availability, being most concentrated in all food categories (Table 1). Flavonoids also have the highest antioxidant potency in vitro, followed by carotenoids, vitamin E and vitamin C. We expect these fundamental differences to shape the relative importance of each antioxidant for consumers as outlined below.

Biochemistry and Tissue Distribution

The biochemistry of antioxidants is fundamental to understanding their possible physiological roles. Biochemistry affects not only the potency (Table 2) but also the mechanism of action (see below) and the tissue

Table 1. Antioxidant content of major food items

Antioxidants	Seeds	Fruits	Leaves	Arthropods*
Vitamin E ¹ (tocopherols and tocotrienols)	0.9 (0.007–1.21)	0.4 (0.1–0.9)	1.3 (0.3–3.1)	0.04 (0.01–0.07)
Carotenoids ² (e.g. β -carotene, lutein, lycopene, canthaxanthin)	0.008 (0.002–0.03)	1.0 (0.0008–2.2)	0.1 (0.02–0.3)	0.2 (0.002–0.4)
Vitamin C ^{3†} (ascorbate and derivatives)	?	18.0 (0.3–113.6)	2.3 (0.2–5.1)	?
Anthocyanins ^{4‡} (e.g. cyanidin 3-glucoside)	1.6 (1.3–1.8)	19.3 (1.3–59.2)	1.0 (0–2.6)	?
Polyphenolic antioxidants (e.g. quercetin, catechin, caffeic acid) ⁵	0.5 (0.5–0.6)	1.4 (0.1–3.2)	34.1 (10.9–105.8)	7.5 (5.4–9.6)

Mean antioxidant concentration in fruits, leaves, seeds and arthropods in $\mu\text{mol/g}$ (range indicated in parentheses). Question marks denote lack of information. Source: (1) Bramley et al. (2000); Marconi et al. (2002); Gomez-Coronado et al. (2004); Konyahoglu et al. (2005); Sivakumar & Bacchetta (2005); Sanchez-Machado et al. (2006); U.S. Department of Agriculture (2007); (2) Hudon (1994); Marconi et al. (2002); Konyahoglu et al. (2005); U.S. Department of Agriculture (2007); (3) U.S. Department of Agriculture (2007); (4) Kähkönen et al. (2001); Czerwinski et al. (2004); Jaakola et al. (2004); (5) Burghardt et al. (2001); Konyahoglu et al. (2005); Määttä-Riihinen et al. (2005).

*The concentration of antioxidants in arthropods is probably dependent on their trophic level, with content decreasing with increasing trophic levels. The data shown here refer only to caterpillars (Lepidoptera) which are in the second trophic level (herbivores). The information available, although probably not representative of all taxa, may still give a gross estimation of the range of variation in antioxidant contents in arthropods relative to that of other food categories. Further studies are needed to investigate the antioxidant content of more arthropod taxa.

†Although vitamin C can be synthesized by many vertebrate taxa (e.g. amphibians, reptiles and most mammals; Chatterjee 1973), a far greater number of species lack this ability, e.g. all teleost fishes (Dabrowski 1990), many birds (Chaudhuri & Chatterjee 1969; Martinez del Rio 1997), and a few mammalian taxa, such as guinea pigs, monkeys, apes and bats (Chatterjee 1973). Therefore, we treat vitamin C here as a regular dietary antioxidant.

‡Owing to their distinctive distribution in plant tissues, anthocyanins are listed separately from other polyphenolic antioxidants. Thus, the sum of both rows indicates total polyphenolic antioxidant contents.

Table 2. Overview of the Trolox equivalent antioxidant capacity (TEAC) values of the most common dietary antioxidants

Family	Antioxidant	TEAC (nM)
Hydrophilic antioxidants		
Vitamin C ¹	Ascorbic acid	0.9
Polyphenols ²	Epicatechin	
	Gallate (F)	4.9
	Quercetin (F)	4.7
	Delphinidin (A)	4.4
	Cyanidin (A)	4.4
	Myricitin (F)	3.1
	Gallic acid (P)	3.0
	Catechin (F)	2.4
Lipophilic antioxidants		
Vitamin E	α -Tocopherol	1.0
Carotenoids ³	Lycopene	2.9
	Cryptoxanthin	2.0
	β -Carotene	1.9
	Lutein	1.5
	Zeaxanthin	1.4
	Astaxanthin	0.03
	Canthaxanthin	0.02

P: phenolic acids; A: anthocyanins; F: other flavonoids. Source: (1) Schofield & Braganza (1996); (2) Rice-Evans et al. (1996); (3) Miller et al. (1996).

allocation of antioxidants. Two broad classes of antioxidants exist: lipophilic antioxidants such as vitamin E and most carotenoids and hydrophilic compounds such as vitamin C and most polyphenolic antioxidants. At the cellular level, hydrophilic antioxidants are found in the cytoplasm, whereas lipophilic antioxidants are found in cell membranes (Halliwell & Gutteridge 2006). At the tissue level, lipophilic antioxidants reach their highest concentrations in liver, adipose tissue, skin and egg yolk (Surai et al. 2000; Packer & Valacchi 2002; Surai 2002), whereas hydrophilic antioxidants are most concentrated in lungs, brain and testes (Kojo 2004; de Boer et al. 2005; Talavera et al. 2005).

A consequence of differential tissue allocation is that measurements of antioxidant activity in one tissue might be unrelated to the antioxidant activity in other tissues, even in the same individual. For example, while in human plasma vitamin C and vitamin E have similar concentrations (30 and 17 $\mu\text{mol/litre}$, respectively), vitamin C is 12–85 times more concentrated than vitamin E in muscles and brain, respectively (Kojo 2004). This is not trivial, given that plasma antioxidant concentration is often used as a stand-alone sample to assess the relevance of an antioxidant for combating oxidative stress.

Mechanisms of Action

Dietary antioxidants reduce oxidative stress by scavenging free radicals by three main mechanisms (variations are possible depending on the reactive species involved). Tocopherols and most polyphenols donate a hydrogen ion, carotenoids quench oxygen singlets and ascorbate transfers electrons (reviewed in Vertuani et al. 2004; Halliwell & Gutteridge 2006). The end result of these actions is that the free radical is neutralized. The efficiency with

which an antioxidant destroys free radicals is called antioxidant potency and is measured in TEAC nM (Trolox equivalent antioxidant capacity; Trolox is a synthetic analogue of tocopherol; see Table 2). After reducing the free radicals, antioxidants are oxidized. At this stage, most antioxidants are fairly stable and relatively innocuous molecules that are subsequently catabolized and excreted. In contrast, carotenoid radicals are rather noxious pro-oxidants that may oxidize other biologically important molecules (Packer et al. 1979; Vertuani et al. 2004). However, if other antioxidants are present (usually vitamin C or E), they usually reduce the oxidized carotenoids, which are thereby recycled (see below). Therefore, the function of carotenoids as antioxidants is debatable and always contingent on the presence of other antioxidants.

Interactions

Because most food items contain several different antioxidants (Table 1), animals commonly absorb a cocktail of antioxidants which might interact positively or negatively (Fig. 1). During absorption, competitive, negative interactions might occur between antioxidants with similar solubility (Fig. 1). Carotenoids and vitamin E, for example, may compete for absorption (Bendich & Shapiro 1986) so that supplementation with high doses of either antioxidant class may result in lower absorption and consequently in lower organismal levels of the other antioxidant class (Bendich & Shapiro 1986; Hageman et al. 1999; Ruiz et al. 1999; Surai 2002). Similar competitive interactions may also occur between flavonoids and vitamin C (Song et al. 2002).

After ingestion, antioxidants found in the same tissues usually interact positively, independent of antioxidant solubility characteristics, as shown in Fig. 1. Typical examples of such interactions are the regenerative actions of vitamins E and C on carotenoids (Mortensen et al. 2001; Amorati et al. 2002) or of polyphenols on vitamins E and C (Pietta & Simonetti 1998; Frank 2005; Fig. 1). Positive interactions between polyphenols and vitamin C are found in both directions, so that either class of antioxidants may regenerate the other, depending on the situation (Pietta & Simonetti 1998; Lotito & Fraga 2000).

Considering interactions among antioxidants is important because it explains, for example, why carotenoids are effective antioxidants only in the presence of vitamins E and C (see above; Palozza & Krinsky 1992; Ruiz et al. 1999) and why supplementation with these two antioxidants may result in greater development of carotenoid-based sexual signals (Pike et al. 2007b). However, further studies are necessary to determine the net outcome of positive and negative interactions for wild consumers and the relevance of such interactions for the expression of various life history traits.

Proximate Mechanisms of the Importance of Antioxidants

There is a fundamental distinction in how antioxidants might affect life history traits. Owing to their tissue

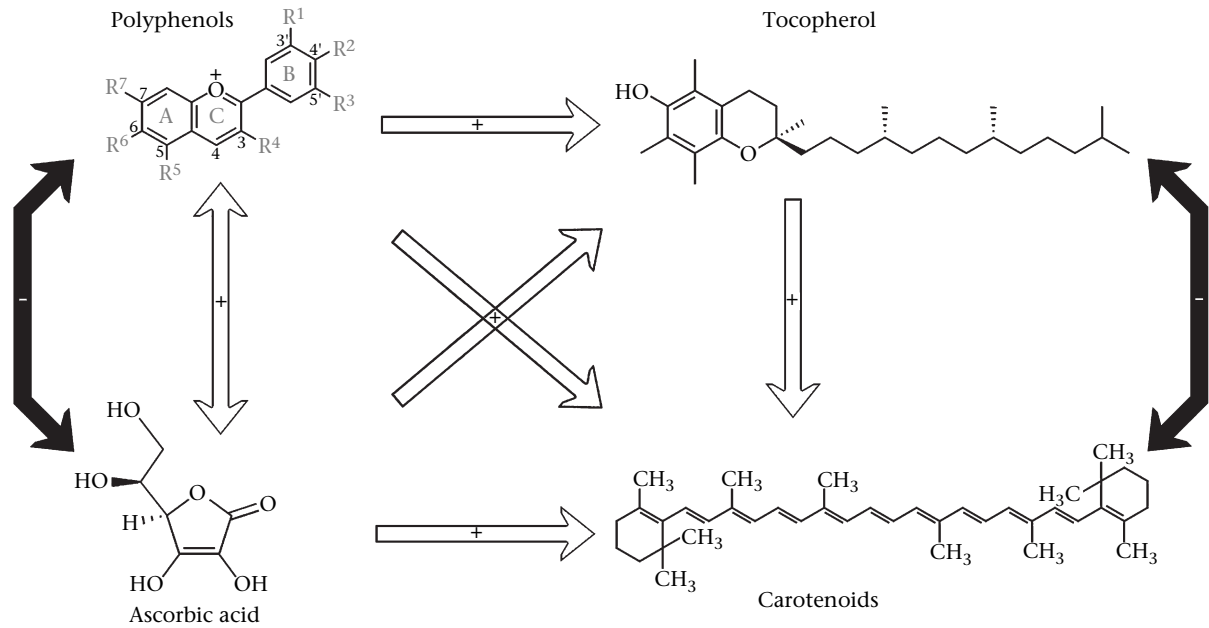


Figure 1. Interactions among the different classes of antioxidants. Negative, competitive interactions occur only during absorption (black arrows). Positive interactions take place between antioxidants present in the same tissues (white arrows). Arrow directions indicate the sense of the interaction, which may also occur in both directions, for example, between polyphenols and vitamin C (see text for further explanations). From Bohm et al. (1997, 1998); Pietta & Simonetti (1998); Lotito & Fraga (2000); Amorati et al. (2002); Kojo (2004); Frank (2005).

distribution and biochemistry, some antioxidants may directly and singly affect the expression of a trait. We term these antioxidants focal antioxidants for a given trait. Examples are lipophilic antioxidants for yolk production and vitamin C for sperm production (see below). Other antioxidants, however, may affect the same trait indirectly, through positive interactions with focal antioxidants or owing to their effects on the overall oxidative status of an individual. The distinction between focal and nonfocal antioxidants is important because the effects of nonfocal antioxidants are contingent upon the presence of focal antioxidants. Such indirect mechanisms probably explain why antioxidants other than carotenoids positively affect the expression of carotenoid-based sexually selected signals (Bertrand et al. 2006; Pike et al. 2007b). An important implication of interactions among dietary antioxidants is that traits can be concomitantly influenced by

different antioxidants. Positive interactions among antioxidants may also increase the overall relevance of common and potent antioxidants, even if these antioxidants are not intrinsically important for a specific trait. Polyphenolic antioxidants, being the most common and potent, are thus likely to be important for a large number of animals and should be incorporated in behavioural studies and those on ecological immunology.

RELATIVE IMPORTANCE FOR LIFE HISTORY TRAITS

Fertility, growth, immunocompetence, ageing/senescence and the development of sexual signals are all life history traits that are affected by the intake of dietary antioxidants (Table 3). Before we review how the various dietary antioxidants may shape various life history traits, it is

Table 3. Relevance of antioxidants for various life history traits

Traits	Vitamin E			Vitamin C			Carotenoids			Polyphenols		
	F	B	M	F	B	M	F	B	M	F	B	M
Fecundity, males	0	+	+	+	+	+	+	0	+	?	?	?
Fecundity, females	?	+	?	?	?	?	0	+	?	?	?	?
Growth	+	+	+	+	±	+		+?		?	?	?
Immunity	+	+	+	+	+	+	+	+	+	?	+?	?
Ageing	?	?	+	?	?	+	?	+?	+	?	?	+
Carotenoid-based sexual signals	+	?	NE	+	?	NE	+	+	NE	?	?	NE
Other sexual signals	?	?	?	?	?	?	?	+	?	?	?	?

F: fish; B: birds; M: mammals; +: trait affected positively by this antioxidant; ±: trait affected only under conditions of extreme stress; +?: trait probably positively affected by these antioxidants, but only little information is available; 0: no effect on this trait for this antioxidant; NE: trait not existent in this class; ?: no studies found. References are given in the main text under the single traits.

important to point out that taxonomic differences in metabolism, physiology and life history may influence the requirements for, and thus the relevance of, specific antioxidants, even for the same trait. For example, taxonomic differences in the effect of vitamin C on various life history traits are linked to differences in species' abilities to produce vitamin C. Veterinary studies have focused on birds and mammals that are able to synthesize ascorbic acid (Chatterjee 1973) and therefore these taxa are probably rarely affected by its exogenous intake. We suggest that the relevance of vitamin C for those birds and mammals that cannot synthesize it (e.g. most passerines, guinea pigs, *Cavia porcellus*, bats and greater primates) may be greater than suggested by studies on livestock.

Fertility and Fecundity

Sperm and, to a lesser extent, oocytes have high metabolic rates, resulting in high production of free radicals (Taylor 2001). Free radicals may damage the sperm membrane, which is rich in polyunsaturated fatty acids and thus extremely vulnerable to oxidation (Blount et al. 2001 and references therein). Furthermore, the DNA of both oocytes and spermatozoa is susceptible to free radical attacks, which may result in both lower fertility and genetic malformation in offspring (Blount et al. 2001). Not surprisingly, therefore, dietary antioxidants have beneficial effects on fertility and fecundity in several animal taxa.

In humans, sperm quality is positively affected by carotenoids, vitamin E and vitamin C (Fraga et al. 1991; Keskes-Ammar et al. 2003; Eskenazi et al. 2005), whereas only carotenoids and vitamin C affect sperm quality in teleost fish (Dabrowski & Ciereszko 2001; Izquierdo et al. 2001; Ahmadi et al. 2006). In fowl, vitamins C and E increase sperm quality, whereas no effect has been found for carotenoids (Monsi & Onitchi 1991; Cerolini et al. 2006; Biswas et al. 2007). Eskenazi et al. (2005) showed how β -carotene, vitamin C and vitamin E may influence different parameters of ejaculate quality in humans. In their study, vitamin C increased sperm count, sperm concentration and total number of motile sperm, and vitamin E influenced sperm motility and the total number of motile sperm, whereas β -carotene showed only a trend towards a beneficial effect on sperm concentration and sperm motility.

In general, vitamins C and E, and carotenoids seem to enhance male fertility in most taxa, albeit through different mechanisms. Vitamin C is likely to be a focal antioxidant during sperm production owing to its high concentration in testes (Fraga et al. 1991). In contrast, vitamin E may be a focal antioxidant during sperm storage, because it may reduce lipid peroxidation of spermatozoa membranes, thereby improving their motility. Finally, carotenoids are probably nonfocal antioxidants affecting male fertility only indirectly. The relevance of polyphenolic antioxidants for male fertility has never been investigated. Since they are mainly hydrophilic, like vitamin C, we hypothesize that polyphenolic antioxidants might improve sperm production.

Compared to male fertility, evidence for the effects of antioxidant supplementation on female fecundity (quantity and quality of oocytes produced) is more conflicting. Although carotenoids are limiting during egg production in lesser black-backed gulls, *Larus fuscus*, (Blount et al. 2004), carotenoid supplementation did not affect egg number in various bird species (Bortolotti et al. 2003; Royle et al. 2003; Remes et al. 2007). Similarly, eggs produced by supplemented females were more likely to hatch and fledge offspring in captive zebra finches, *Taeniopygia guttata* (McGraw et al. 2005) but not in free-ranging great tits, *Parus major* (Remes et al. 2007). Similarly, carotenoid supplementation did not affect egg number (Choubert et al. 1998) but increased the rate of fertilization and percentage of hatched eggs in rainbow trout, *Oncorhynchus mykiss* (Ahmadi et al. 2006). Finally, vitamin E supplementation resulted in heavier and more fertile eggs in chickens, *Gallus gallus domesticus* (Muduuli et al. 1982), while vitamin C increases fertility and embryo survival in teleost fish and guinea pigs (Habibzadeh et al. 1986; Dabrowski & Ciereszko 2001).

Owing to their high concentrations in yolk, we suggest that carotenoids and vitamin E are focal antioxidants for egg production. Further studies are needed to corroborate the effect of vitamin E on egg quality in fish. In contrast, vitamin C does not accumulate in egg yolk and thus probably contributes only indirectly to female fecundity. Note, however, that, except for monotremes, egg production is unlikely to be costly in mammals as they produce only a limited number of small eggs with no or small amounts of yolk. Moreover, oxidative stress may negatively affect placental function and fetal growth (Myatt & Cui 2004). While it is known that antioxidants pass the human placenta (van der Heide et al. 2003; Herrera et al. 2004; Myatt & Cui 2004), no information is available about the relevance of single antioxidants for fetal development.

Growth

The high metabolic rate required for tissue growth produces large amounts of free radicals (Rollo 2002) that may eventually result in reduced life span (Rollo et al. 1996; Metcalfe & Monaghan 2003). Since a faster growth rate may reduce juvenile mortality (Abrams et al. 1996) and increase reproductive output (Metcalfe & Monaghan 2003), the ingestion of dietary antioxidants may help to counter negative effects of oxidative stress associated with fast growth rate.

In contrast to carotenoids, vitamins E and C improve growth rates in teleost fish and mammals (Cromwell et al. 1970; Stephens et al. 1979; Rehulka 2000; Sealey & Gatlin 2002). In poultry, vitamin E is reported to improve growth rate, while the effects of vitamin C on growth are restricted to incidences of very high overall stress (i.e. high temperatures or population density; Abdel-Raheem & Ghaffar 2004; de Ayala et al. 2006). Very little information is available on the effect of carotenoids on chick growth, but Cucco et al. (2006) reported improved growth in grey partridge, *Perdix perdix*, chicks supplemented with β -carotene.

Because growth is not restricted to a single tissue, it is probably mainly affected by the overall oxidative status of an individual. Following this conjecture, we predict that those antioxidants with the highest antioxidant potency and environmental abundance, that is, polyphenolic antioxidants, would play an important role during growth.

Immunocompetence

The activation of the immune system generates large amounts of free radicals which, in turn, may undermine its functioning (e.g. Halliwell & Gutteridge 1985; Gruner et al. 1986; von Schantz et al. 1999). A high intake of dietary antioxidants may thus enhance immunocompetence by alleviating its side-effects. Furthermore, some antioxidants have coenzymatic activity and may influence cell-to-cell communication, thereby modulating some branches of the immune system (e.g. Middleton 1996; Hughes 1999a, b).

Carotenoids, vitamin C and vitamin E can all enhance both specific and nonspecific immune responses in several species of fish, bird and mammals (Tanaka et al. 1979; Amar et al. 2000; Ortuño et al. 2000; Sahoo & Mukherjee 2002; Abdel-Raheem & Ghaffar 2004; Chew & Park 2004; McGraw & Ardia 2004; Lohakare et al. 2005). Flavonoids have direct effects on a variety of immune and inflammatory cell functions in vitro, particularly once cells are activated (Middleton & Kandaswami 1992), with both inhibitory and stimulatory actions (Middleton et al. 2000). Beneficial effects of flavonoids in vivo were recently demonstrated in a passerine bird species, where supplementation enhanced humoral immune responsiveness (Catoni et al., in press).

Since all antioxidants affect the immune system, we suggest that the relevance of single antioxidants is mainly determined by antioxidant intake and uptake. That is, the antioxidant that is most ingested (and absorbed) is the most relevant for a given individual at a moment, and no generalizations can be made.

Ageing and Senescence

Ageing is defined as the progressive malfunctioning of cellular and organismal functions with the advancing of age which may eventually cause death (Finkel & Holbrook 2000). In evolutionary ecology, ageing is defined as 'senescent declines in fitness components [...] resulting from a lack of investment in some underlying maintenance trait' (Williams et al. 2006, page 5). It is commonly accepted that endogenous reactive species are responsible for the accumulation of irreversible nuclear and mitochondrial DNA mutations (Balaban et al. 2005), which result in mitochondrial dysfunctions and overall physiological decline. Because this decline is central to the process of senescence (Beckman & Ames 1998), dietary antioxidants are presumably important for lowering oxidative stress and slowing down the rate of ageing.

Experimental supplementation of antioxidants successfully increased life span in fruit flies and beetles (Garg &

Mahajan 1994; Kakkar et al. 1996; Driver & Georgeou 2003), but it failed to produce significant effects in mice, *Mus musculus*, or humans (Holloszy 1998; reviewed in Golden et al. 2002; Selman et al. 2006). Nevertheless, several studies have shown that various antioxidants may improve specific, senescence-related pathologies. Flavonoid and vitamin E supplementation successfully retarded, reduced in severity or even reversed degenerative brain diseases in mice and humans (Rai et al. 1991; Patil et al. 2003; McDonald & Forster 2005; Sun et al. 2007). Similarly, vitamin E, vitamin C, carotenoids and polyphenols successfully retarded the onset or reduced the severity of arthritis in mice and humans (Haqqi et al. 1999; Darlington & Stone 2001; Karatas et al. 2003). Given the focus on human-typical diseases, the relevance of these studies for animals is unknown.

Sexual Signal Development

High metabolic rates associated with the expression of exaggerated sexually selected traits also lead to an increased production of free radicals. Oxidative stress has thus been identified as a fundamental agent linking sexually selected signals to the underlying individual quality of the sender (von Schantz et al. 1999). Correlations between individual quality, oxidative stress and expression of carotenoid-based ornaments have subsequently been found (e.g. Blount et al. 2003; Faivre et al. 2003; Saks et al. 2003; Pike et al. 2007a; but see Isaksson & Andersson 2008). Since carotenoid-based ornamentation can be enhanced by vitamin C and vitamin E in fish (Pike et al. 2007b), it is also a prime example of interactions among antioxidants. Such interactions might also occur with nondietary antioxidants because melatonin, a hormone with antioxidant characteristics, enhanced carotenoid coloration in a passerine (Bertrand et al. 2006). We suggest that similar positive interactions occur between carotenoids and polyphenolic antioxidants for which no information is currently available.

Information on the effect of dietary antioxidants on the expression of sexually selected traits other than carotenoid-based ornaments is scant. Dietary antioxidants may influence animal colouration because most pigments produced by vertebrates and invertebrates (e.g. melanin) have antioxidant properties (McGraw 2005). Some dietary antioxidants might replace pigments in their antioxidative roles and consequently increase their availability for pigmentation. So far, no studies appear to have tested this conjecture.

Acoustical sexual displays could also be sensitive to oxidative stress and thereby affected by the intake of dietary antioxidants, since developmental stress can affect relevant brain structures, song complexity, attractiveness and female choice in zebra finches as well as song quality in starlings, *Sturnus vulgaris* (Buchanan et al. 2003; Spencer et al. 2003, 2005). Moreover, exaggerated morphological signals may also be sensitive to antioxidant status (von Schantz et al. 1999) and thereby be improved by the intake of dietary antioxidants. Indeed, carotenoid supplementation resulted in increased length of sexually

selected tail feathers in pheasants, *Phasianus colchicus* (Smith et al. 2007).

EFFECTS OF ECOLOGY AND BEHAVIOUR ON ANTIOXIDANT INTAKE

So far, we have emphasized that the intrinsic relevance of different dietary antioxidants may vary among taxa and life history traits according to antioxidant chemistry and the physiology of a given trait (Table 3). For free-ranging consumers, however, the environmental availability of antioxidants and diet choice are two crucial factors that may affect the specific relevance of antioxidants. Unfortunately, both factors have as yet received little attention in behavioural studies and evolutionary ecology.

Environmental Variability in Antioxidant Availability

The environmental availability of food varies spatially and temporally (Fig. 2), even in areas with seemingly stable climate such as tropical forests (Leighton & Leighton 1983; Wolda 1988). Consequently, the quantity and quality of antioxidants available to a consumer also vary temporally and spatially. Temporal variation entails that the relevance of an antioxidant for a trait is, at least partly, contingent on the duration of expression of this trait.

Traits such as fertility, growth or the expression of sexually selected traits occur mostly during relatively short, predictable periods of the year. Within a geographical location, these traits are likely to be consistently influenced by the same dietary antioxidants. Ageing and immunity are, however, continuous traits and therefore prone to seasonal variation in antioxidant availability. Trade-offs involving immunity or, more generally, self-maintenance are thus unlikely to be primarily affected by a single dietary antioxidant throughout the year. For example, immunity and self-maintenance in omnivorous birds may be mainly affected by vitamin E and carotenoids during spring and early summer, when they feed on arthropods. However, from late summer to winter many species feed on the abundant fruit supply, and the same traits may be affected by the large amounts of ingested polyphenolic antioxidants and vitamin C.

The importance of spatial variability in antioxidant availability has been addressed only in the context of carotenoid-based signalling. Geographical differences in carotenoid availability result in population-specific expression of sexually selected carotenoid-based ornaments in guppies, *Poecilia reticulata*, and house finches, *Carpodacus mexicanus* (Grether et al. 1999; Hill et al. 2002). Thus, pronounced geographical variability in carotenoid availability translates into differences in the development of sexually selected signals (Hill 1993) and may limit a direct comparison of individual quality among populations or

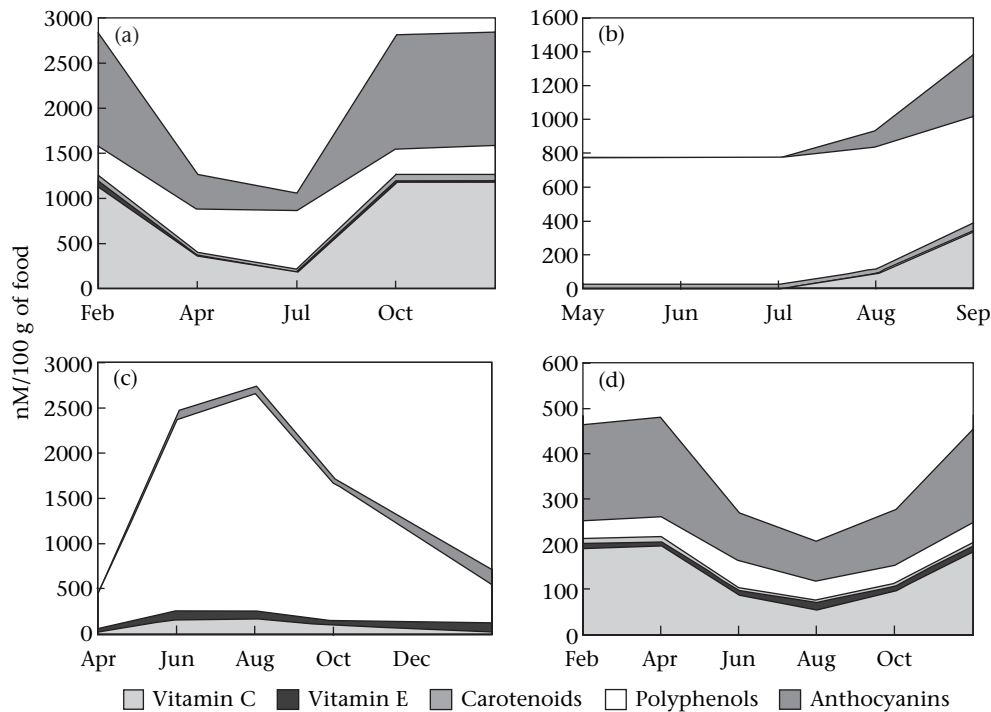


Figure 2. Omnivorous species show dramatic (up to five-fold) seasonal variation in antioxidant intake. This is exemplified by a selection of very different, free-ranging animals that differ in diet, life history and habitat type: (a) European badger, *Meles meles*; (b) ocellated lizard, *Timon lepidus*; (c) prairie chicken, *Tympanuchus cupido*; (d) orange-rumped agouti, *Dasyprocta leporina*. We selected these animals because they differ strongly in diet and detailed, seasonal information on their diet is available. We multiplied the intake of different food categories, seeds, fruits, leaves and arthropods, by their mean antioxidant contents (Rumble et al. 1988; Hodar et al. 1996; Henry 1999; Rosalino et al. 2005). We performed these calculations not to obtain exact values of antioxidant intake, but rather to illustrate the magnitudes of temporal variation in the intake of different antioxidants within species. Note that axis scales differ between species.

even within migratory populations that moult in different localities (Norris et al. 2006). Even more importantly, the availability of carotenoids translates also into differences in mate preferences (Grether et al. 2005). This is a crucial process, because spatial variability in carotenoid availability may lead to different geographical patterns in the expression of a phenotypic trait that may be important for population divergence based upon sensory drive (Fuller et al. 2005).

Food Choice

Antioxidant concentrations differ up to five orders of magnitudes both within and between food categories (Table 1). Food selection is thus important for determining the intake of antioxidants. If animals select food items that have high contents of rare antioxidants, they may mitigate the severity of the constraints outlined above.

Food selection is a fundamental process that has been documented in most organisms. It has been mainly related to differences in macronutritional contents in optimal foraging theory. Surprisingly, although fundamental for our understanding of the role of carotenoids in evolutionary ecology, no study has investigated the hypothesis that animals might select carotenoid-rich food (e.g. Olson & Owens 1998). Recently, however, Schaefer et al. (2008) and Catoni et al. (in press) have shown that birds select food rich in anthocyanins and flavonoids, respectively, if given a choice between two otherwise identical foods.

The proximate mechanisms of distinguishing between foods with different antioxidant contents may vary. Antioxidants that function as pigments, such as anthocyanins and carotenoids, may be detected visually. In fruits, however, only anthocyanins are visually discernible because they usually mask the contents of carotenoids owing to their higher concentrations (Schaefer et al. 2008). To date, no study has investigated whether the concentration of carotenoids or polyphenolic antioxidants is visually discernible in seeds or arthropods. Like other micronutrients, nonpigmentary antioxidants may be selected by means of taste or physiological feedbacks (e.g. Fromentin & Nicolaidis 1996; Pierce & McWilliams 2005).

A plausible but still untested idea is that interindividual differences in the ability to discern antioxidants, or food quality in general, might result in large differences in antioxidant intake. Differences in antioxidant intake may arise as a by-product from food selection directed at nutrients. Such interindividual differences in food selection have been demonstrated for a broad range of taxa in captivity, and may well be present in free-ranging animals (e.g. Carciofi et al. 2006; Nowakowski et al. 2006). The ability to select food rich in antioxidants might be particularly important during periods of sickness. Several species of mammals and birds select food for self-medication to reduce noxious effects of target parasites and toxins (Clayton & Wolfe 1993; Huffman 1997; Lozano 1998). Thus far, the concept of 'nutraceutical' self-medication has not been applied to dietary antioxidants and it would be easily

overlooked, unless it is specifically taken into consideration in experimental set-ups.

FUTURE RESEARCH DIRECTIONS

To develop a more complete framework for the relevance of dietary antioxidants for the expression of life history traits and trade-offs, we suggest that future studies should consider the full range of available antioxidants, their possible interactions, their environmental availability, and the potential for interindividual differences in antioxidant intake and uptake. This is because more dietary antioxidants than traditionally perceived may affect crucial life history traits, such as fertility, growth, immunity, senescence and the expression of sexually selected traits. Surprisingly, the most common and potent antioxidants, flavonoids and phenolic acids, are those that have received the least attention. Given their ubiquity in food items, we urge researchers to include these potentially important antioxidants in evolutionary ecology as well as ecological immunology studies.

Since antioxidants differ in their chemistry and tissue distribution, they may not be equally relevant for each trait, and we have highlighted one fundamental distinction between antioxidant types: those that act directly upon traits and those that affect traits indirectly. To date, the concept that the relevance of single antioxidants for various life history traits is likely to differ has hardly been accounted for in evolutionary ecology. To address this concept we suggest that the absorption and tissue distribution of several antioxidant classes should be studied in different animal taxa. In addition, to establish the relevance of single antioxidants for given life history traits, the effect of supplementation should be compared for different kinds of antioxidants (similar to Eskenazi et al. 2005). Experiments should also ideally use different mixtures of antioxidants, mimicking a more natural situation, to account for interactions among antioxidants (e.g. the relevance of carotenoids in the presence of high concentrations of flavonoids or vitamin C).

Owing to the large differences of antioxidant contents between food items, animals might select food to optimize their intake of specific antioxidants or antioxidants in general. To assess the relevance of food selection, the environmental availability of antioxidants can be compared with the actual intake of antioxidant in free-ranging animals. Selection for increasing antioxidant intake can be unequivocally demonstrated in captivity by measuring the consumption of various otherwise identical food types differing in antioxidant content (similar to Schaefer et al. 2008; Catoni et al., in press). By modifying visual and taste cues between food types, we can establish the proximate mechanisms that animals use to discern antioxidants in food.

Environmental variation in the availability of antioxidants and individual variation in food selection may result in interindividual differences in antioxidant intake that may explain, at least partially, intra- and interspecific differences in resource allocation to self-maintenance or reproduction (e.g. Martin et al. 2004; Ardia 2005; Møller

et al. 2006). A corollary of this conjecture is that the differences in optimal allocation of resources to either reproduction or self-maintenance may not only be determined genetically, but may also be dependent on environmental factors and individual behaviour. We suggest that interindividual differences in food selection may limit the interpretation of many relatively controlled experiments. Individuals in the same experimental group fed with an array of different food items (e.g. a mixture of different seeds) might differ in food selection and consequently food, and antioxidant, intake. These interindividual differences might augment the within-group variance and diminish the between-groups differences in antioxidant intake, thereby limiting our understanding of the relevance of antioxidant supplementation. Importantly, if we do not consider the magnitude and the consequences of environmentally and behaviourally induced variations in antioxidant intake, we are not able to determine the importance of the genetic component. Therefore we suggest that controlled studies should either measure the intake of each food item separately (e.g. McGraw et al. 2003) or reduce the possibilities of food choice by using homogeneous or semisynthetic food with exactly quantified composition (e.g. Pierce et al. 2005).

Concluding, we predict that analysis of the interplay between environment and behaviour (food selection) will generate exciting insights into intraspecific (e.g. between sexes) and interspecific differences in the relevance of antioxidants for the expression of life history traits. Based on this knowledge, we will be better able to evaluate the significance of dietary antioxidants in determining the severity of life history trade-offs.

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