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Good genes, oxidative stress and condition-dependent sexual signals

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The immune and the detoxication systems of animals are characterized by allelic polymorphisms, which underlie individual differences in ability to combat assaults from pathogens and toxic compounds. Previous studies have shown that females may improve offspring survival by selecting mates on the basis of sexual ornaments and signals that honestly reveal health. In many cases the expression of these ornaments appears to be particularly sensitive to oxidative stress. Activated immune and detoxication systems often generate oxidative stress by an extensive production of reactive metabolites and free radicals. Given that tolerance or resistance to toxic compounds and pathogens can be inherited, female choice should promote the evolution of male ornaments that reliably reveal the status of the bearers' level of oxidative stress. Hence, oxidative stress may be one important agent linking the expression of sexual ornaments to genetic variation in fitness-related traits, thus promoting the evolution of female mate choice and male sexual ornamentation, a controversial issue in evolutionary biology ever since Darwin.

Keywords: allelic variation; detoxication; major histocompatibility complex; oxidative stress; sexual ornaments

1. INTRODUCTION

There are numerous suggestions as to what females can gain by being selective in their choice of mate (Fisher 1915; Kirkpatrick & Ryan 1991; Andersson 1994). Females can gain direct benefits, such as essential territorial resources, paternal care or avoidance of infectious diseases, by mating with healthy males with large and conspicuous ornaments such as bright colours and elongated plumes (Kirkpatrick & Ryan 1991). When no direct benefits are at hand, Fisher's (1958) runaway process (reviewed in Andersson 1994) suggests that an association between alleles for a larger ornament and alleles for the female preference will arise merely owing to the female mating preference. The good-genes models suggest, on the other hand, that males differ in condition and viability and that such traits can be inherited by their offspring. Females can assess this variation in male genetic quality if males in better condition express more exaggerated ornamental traits (Fisher 1915, 1958; Zahavi 1975; Hamilton & Zuk; 1982; Kodric-Brown & Brown 1984; Andersson 1986).

The condition-dependence of male ornaments is vindicated by studies showing that the expression of traits, such as tail ornaments and combs in birds, and carotenoid pigmentation in fishes and birds, correlates with condition and survival (Andersson 1994). Experiments with controlled infections show that ornaments are more sensitive to diseases than are other morphological traits (Zuk *et al.* 1990; Houde & Torio 1992; Møller 1994). The good-genes models are specifically supported by recent studies showing that female birds can increase offspring fitness by mating with more ornamented males without obtaining any direct benefits (Norris 1993; Møller 1994; Petrie 1994; von Schantz *et al.* 1994; Hasselquist *et al.* 1996).

There have been only a few attempts to identify polymorphic genes that confer variation on both fitness traits and ornamental expression (Watt *et al.* 1986; von Schantz *et al.* 1996). By using the often detailed knowledge of molecular and physiological actions of genes with major effects on health and condition, we hope to encourage future studies on the evolution of condition-dependent sexual ornaments and other traits closely linked to fitness. We believe that evolutionary biologists can learn from immunologists and toxicologists, who have long been aware of the remarkable polymorphism in the genetic systems that govern an organism's immune response and excretion of toxic compounds. Allelic variation at many of these loci clearly affects health (reviewed in Gonzalez & Nebert 1990; Nebert *et al.* 1996; Kalow 1997; Apanius *et al.* 1997). Although many other genes may affect an individual male's condition and ornamentation, we focus on the effects of the genes involved in immune defence and processing of toxic compounds. These genes are fairly well studied and have a broad taxonomic distribution. Moreover, these defence systems seem to confer an unusually strong interaction between individual genotypes and the environment (i.e. local pathogens and toxins) and, hence, a potential for maintained genetic variation for fitness-related traits.

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Table 1. *Summary of data on genetic variation in various immune and biotransfomation gene families in humans*

	supergene family	number of gene families	number of variable loci (total number of loci)	maximum no. of alleles found at one locus (name of locus, and if available number of alleles found among Caucasians)	additional variation
immune genes					
	MHC class I[a]	1	5 (6)	149 (*HLA-B*, $\geqslant$50)	
	MHC class II A and B[a]	2	8–9 (8–10)	179 (*HLA-DRB*, $\geqslant$31)	heterodimer formation
biotransformation genes					
phase I	cytochrome P450 (*CYP*)[b]	14	9 ($\geqslant$36)	53 (*CYP2D6*, 53)	
	alcohol dehydrogenase (*ADH*)[c]	1	4 (7)	3 (*ADH2*)	heterodimer formation
	aldehyde dehydrogenase (*ALDH*)[d]	1	3 (10–12)	3 (*ALDH9*)	
	NAD(P)H-quinone oxidoreductase (*NQO*)[e]	1	2 (2)	'highly polymorphic' (*NQO2*)	alternative splicing
phase II	glutathione-*S*-transferase (*GST*)[f]	5	4 (>19)	3 (*GSTM1*)	heterodimer formation
	sulphotransferase (*ST*)[g]	1	2 (5)	3 (*STP1*, *STP2*)	alternative splicing
	methyltransferase (*MT*)[h]	1	3 (3)	3 (*TPMT*)	
	N-acetyltransferase (*NAT*)[i]	1	2 (2)	15 (*NAT2*, $\geqslant$9)	

[a] Grubic *et al*. 1995; Trowsdale 1995; Parham & Ohta 1996; Zacharay *et al*. 1996.
[b] Cohen *et al*. 1992; Nakagawa *et al*. 1993; Clot *et al*. 1994; Nebert *et al*. 1996; Nelson *et al*. 1996; Parkinson 1996; Marez *et al*. 1997.
[c] Burnell *et al*. 1987; Edman & Maret 1992; Zgombic-Knight *et al*. 1995.
[d] Goedde *et al*. 1992; Hsu *et al*. 1997; Yoshida *et al*. 1998.
[e] Jaiswal *et al*. 1990; Rosvold *et al*. 1995; Yao *et al*. 1996.
[f] Pemble *et al*. 1994; Hayes & Pulford 1995; Nelson *et al*. 1995; Yengi *et al*. 1996.
[g] Dooley & Huang 1996.
[h] Lachman *et al*. 1996; Parkinson 1996; Tai *et al*. 1996.
[i] Vatsis *et al*. 1995.

To assure the condition-dependent expression of the ornament it should impose a handicap (Zahavi 1975) or cost to the bearer that increases with ornamental expression (Andersson 1986). The currencies mediating this cost function are suggested to be in terms of energy trade-offs or increased predation rates (Andersson 1994). To identify the physiological burdens underlying ornamental development and maintenance, we suggest that the condition-dependent costs need not to be carried to such ultimate terms as energy reallocations and altered chances of death by predation. Extensive empirical data at the cellular level show that oxidative metabolites and free radicals, which are highly reactive by-products of normal metabolism and immune defence, cause extensive oxidative damage to DNA, proteins and lipids; this process is known as oxidative stress (Burton & Ingold 1984; Halliwell & Gutteridge 1985; Gruner *et al*. 1986; Breimer 1990; Kappus 1993; Gregus & Klaassen 1996; Parkinson 1996). Oxidative stress appears to be a major contributor to ageing and to various degenerative diseases such as cancer and immune and brain disorders (reviewed in Coyle & Puttfarcken 1993; Frei 1994; Ahmad 1995; Sies 1997).

Free radicals are generated particularly by mitochondrial respiration and by the operation of the genetically polymorphic immune and biotransformation systems (Klebanoff & Clark 1978; Halliwell & Gutteridge 1985; Anderson & Theron 1990; Shigenaga & Ames 1994; Nebert *et al*. 1996; Parkinson 1996). The costs induced by oxidative stress may hence be a reliable currency in the trade-off between individual health and condition-dependent ornamental sexual traits, the expression of which in many cases appears to be particularly sensitive to oxidative stress.

2. GENETIC VARIATION AND FUNCTION OF THE IMMUNE AND DETOXICATION SYSTEMS

The immune system (Roitt *et al*. 1996) and the detoxication system (Gregus & Klaassen 1996) in animals have many characteristics in common. Both systems aim to identify foreign compounds and to destroy pathogens or excrete toxic substances with high specificity. Harmful pathogens and toxins occur in a great variety in nature and an individual's ability to fight an assault depends on its capacity to identify alien compounds and trigger an appropriate response. These defence systems are characterized by a high genetic diversity and complexity at loci that code for antigen or substrate affinities (table 1). All genetic complexes denoted in table 1 are involved in the recognition of and defence against foreign

compounds. Each gene family often contains several loci, some of which have a substantial allelic polymorphism (table 1) known to affect various fitness-related traits (Gonzalez & Nebert 1990; Nebert *et al*. 1996; Kalow 1997; Apanius *et al*. 1997). Their operation may therefore be especially relevant to the good-genes process of sexual selection.

The good-genes process has been questioned; however, several extant theoretical studies elucidate how and why genetic polymorphisms can evolve and be maintained. As concerns the immune system, most arguments for the maintance of allelic polymorphism are based on overdominance (Hughes & Nei 1988), evasion–detection races between parasite and host, and frequency-dependent selection (Hamilton & Zuk 1982; Eshel & Hamilton 1984; Hamilton *et al*. 1990; Apanius *et al*. 1997). In general it appears that multilocus systems coding for proteins with overlapping functions (table 1) facilitate the persistence of allelic polymorphism (Hamilton *et al*. 1990; Kirzhner *et al*. 1996; Nevo *et al*. 1997). In addition, for positive parent–offspring correlations to exist, as is essential for the good-genes process (Hamilton & Zuk 1982), the polymorphisms must be dynamic (Dieckmann & Law 1996; Kirzhner *et al*. 1996) owing to constantly changing selective regimes such as those characterizing host–parasite interactions (Eshel & Hamilton 1984; Hamilton *et al*. 1990).

(a) *The adaptive immune system*

MHC (major histocompatibility complex) molecules bind antigens from pathogens and present them to T-cells. The antigen-binding properties of the MHC molecules, which differ according to the particular MHC alleles an individual carries, determine which foreign peptides can be identified for triggering a specific, adaptive, immune response (Roy *et al*. 1989). The allelic polymorphism of the MHC is extraordinary; in humans there are now more than 170 different alleles identified at one MHC locus (table 1). Several studies have identified different MHC alleles and haplotypes that confer resistance to various infectious diseases and autoimmune disorders in humans and domesticated birds and mammals (Apanius *et al*. 1997).

The MHC antigen presentation to T-cells is necessary to trigger the synthesis of antigen-specific antibodies and to establish an immunological memory that will protect the individual from re-infections with the same pathogen (Clark & Ledbetter 1994; Rajewsky 1996; Roitt *et al*. 1996; Zinkernagel 1996).

(b) *Detoxication*

The biotransformation enzymes participate not only in the metabolism of naturally occurring chemicals, such as secondary plant metabolites and toxins in ingested plants, fungi and animals, but also in the metabolism of various artificial chemicals and drugs (Gregus & Klaasen 1996). Xenobiotic metabolism is typically divided into phase-I (functionalization) and phase-II (conjugation) reactions. Phase-I enzymes, for example the cytochrome P450s (CYP) (table 1), catalyse the incorporation of a functional group (-OH, -NH_2, -SH or -COOH) into the initially hydrophobic substrate. Phase-II enzymes, for example glutathione-*S*-transferases (GST) (table 1), make the molecule less reactive by conjugation of the functional group with glutathione, sulphate or glucuronic acid. These reactions generally make the substrate water-soluble, and the conjugated endogenous compound further facilitates the excretion of the product (Hayes & Pulford 1995).

Various biotransformation enzymes exhibit overlapping substrate specificities (Gonzalez & Nebert 1990). Still, different enzymes differ in their capacity to detoxify a given chemical and allelic variants can affect the individual's ability to metabolize different compounds (Gonzalez & Nebert 1990; Daly *et al*. 1993; Nebert *et al*. 1996; Tai *et al*. 1996; Kalow 1997). In humans, the frequency of poor and extensive metabolizers of various drugs differs between ethnic groups to a far greater extent than can be expected from rare mutation events or genetic drift (Gonzalez & Nebert 1990; Nebert *et al*. 1996).

Among the biotransformation genes, only *CYP* and *NAT* show exceptionally high allelic polymorphism (table 1); this may in part be due to the extensive amount of research that has been directed towards these loci (Gonzalez & Nebert 1990; Nebert *et al*. 1996; Kalow 1997). In addition, the nature of a species' diet may have an effect on the allelic polymorphism of particular biotransformation genes. For example, the human alcohol dehydrogenase (*ADH*) genes have only a minor allelic polymorphism (table 1), whereas the *ADH* locus has an extensive allelic variation in *Drosophila* species (McDonald & Kreitman 1991; Stam & Laurie 1996), which often feed on various fermented plant products. Despite the marked difference in allelic polymorphism between the immune and detoxication genes (table 1), it seems likely that the polymorphism in both cases is maintained by similar processes. Much of the redundancy and complexity of the biotransformation system of animals may have evolved as a response to chemical defences in ingested plants, animals, bacteria and fungi (Gonzalez & Nebert 1990; Kalow 1997). At least in plants, the arrays of such chemicals are highly variable both within and among species (Fritz & Simms 1992). Both temporal and spatial variations can therefore confer dynamic evolutionary interactions between plants and herbivores (Berenbaum & Zangerl 1998), similar to those between parasites and hosts (Eshel & Hamilton 1984; Hamilton *et al*. 1990), which can help to maintain the allelic polymorphism observed in those biotransformation enzymes that metabolize exogenous compounds.

Some of the biotransformation enzymes also catalyse the metabolism of endogenous compounds, such as steroid hormones (Parkinson 1996). Interestingly, many secondary plant metabolites are steroid-like compounds (Beier 1990; Knight & Eden 1995) likely to interfere with herbivores' physiology. As a potential countermeasure the consumers' biotransformation enzymes are likely to evolve to escape the negative effects of exogenous steroid-like toxins. In insects, variation in insecticide resistance is frequently associated with polymorphic genes regulating the transcription of CYP and GST enzymes (Grant & Hammock 1992; Feyereisen *et al*. 1995). The most extensively studied inducer in vertebrates is the Ah receptor, which binds certain dioxin-related compounds and initiates the transcription of a battery of individual

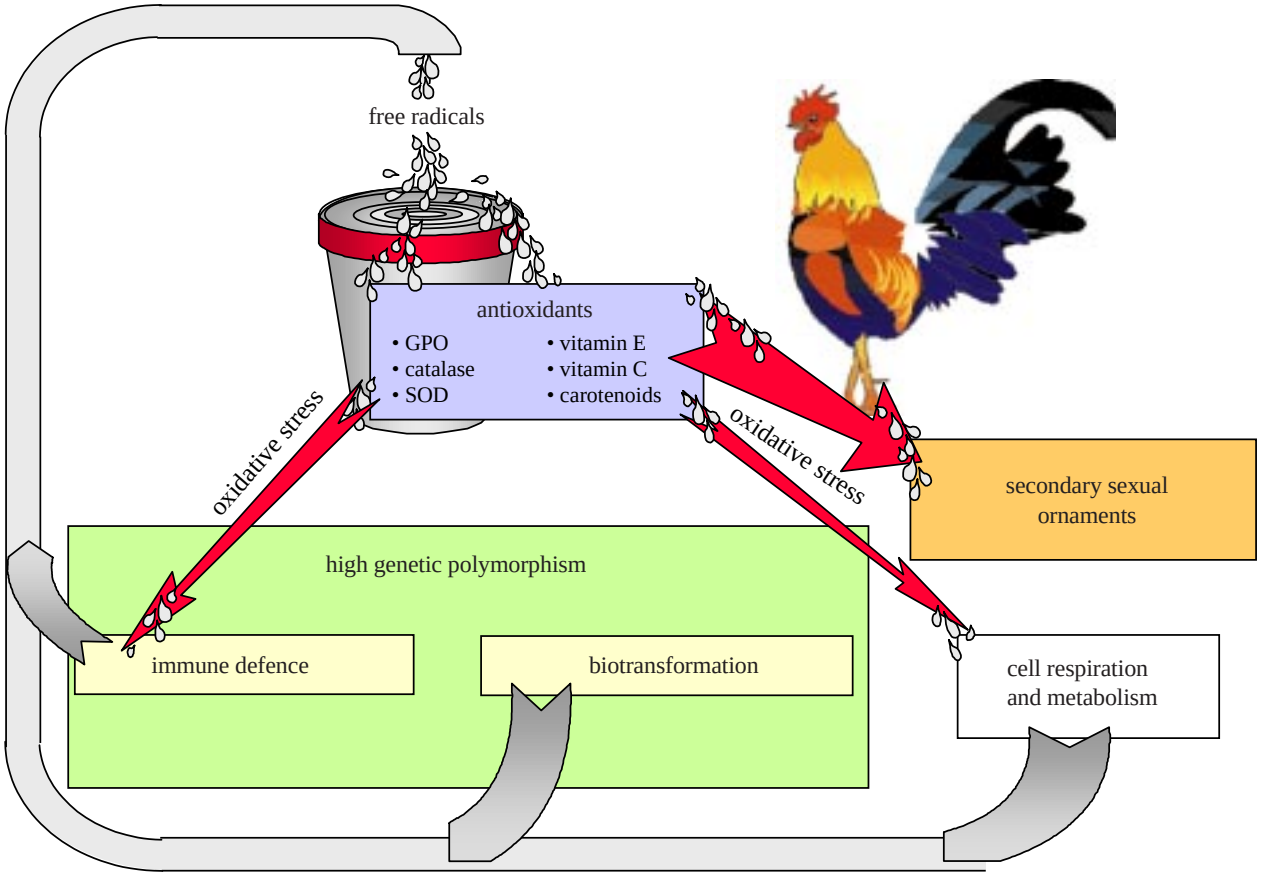

Figure 1. Schematic representation of the pathways linking the load of oxidative stress to the expression of condition-dependent sexual ornaments via activated defence systems. The figure ignores cases where the toxin or pathogen overcomes the defence systems and causes direct cell dysfunction and death.

An individual's exposure to oxidative stress is suggested to be an important mediator of condition-dependent ornamentation. The contents of the central pot in the figure represent the load of reactive metabolites and free radicals produced by cell respiration and metabolism, biotransformation and immune defences. At some baseline level the reactive intermediates and free radicals are destroyed by various antioxidants (purple box); this prevents leakage of oxidants and tissue damage. Different types of antioxidant can act complementarily to each other so that the reaction of one can spare others and to the extent that one antioxidant becomes depleted, the deposits of others may be reduced. Hence some sexual ornaments, such as carotenoid pigmentation in plumage or skin, known to be condition-dependently expressed in birds and fishes, may lose their hue even before any cytotoxic effects of oxidative stress occur: that is, before the load of free radicals starts to flow over the edge of the pot in the figure.

Excessive production of free radicals can be generated not only by cell respiration but also by activated immune defences and biotransformation systems. These defence systems are characterized by a high level of genetic polymorphism (green box).

The expression of various secondary ornaments, such as plumes, spurs, combs and song repertoire sizes in birds, appears to be particularly sensitive to oxidative stress. To the extent that these traits are more susceptible to oxidative stress than are vital functions, the ornaments can serve as external cues for females to select particularly resistant or tolerant males, even before any pathological disorders appear.

phase-I and -II enzymes (Nebert *et al.* 1996). In mice the Ah receptor is encoded by a single gene with four different alleles (Poland *et al.* 1994) that confer differences in the bearers' susceptibility to various disorders (Nebert *et al.* 1996) and exposure to dioxin (Nebert *et al.* 1972).

3. OXIDATIVE STRESS

The immune and the detoxication systems have another important attribute in common: when activated they are generating reactive metabolites and free radicals, which contribute to an individual's level of oxidative stress (figure 1).

Ultimately, energy in terms of ATP is essential for all bodily functions. However, the formation of ATP, fuelled by oxidative metabolism in the mitochondria, generates free radicals (Halliwell & Gutteridge 1985; Coyle & Puttfarcken 1993; Packer *et al.* 1994). Free radicals are atoms or molecules that contain one or more unpaired electrons (Leffler 1993); these unpaired electrons make them very prone to react with other molecules. Reactive radicals, and especially the hydroxyl radical (OH·), can damage a variety of critical molecules and physiological processes, including DNA, proteins, lipids, cell membranes, expression of MHC class-II molecules and suppression of both T- and B-cell-based immune reactions (Halliwell & Gutteridge 1985; Gruner *et al.* 1986; Breimer 1990). There are a number of experimental studies on rodents (reviewed by Youngman *et al.* 1992; Shigenaga & Ames 1994) showing that dietary restrictions of calorie

intake not only cause a delay in reproduction and decreasing cell proliferation rates and body growth but also significantly increase longevity. These effects are associated with reduced mitochodrial respiration and reduced oxidative damage to various molecules and organs (Youngman *et al.* 1992; Shigenaga & Ames 1994).

The production of free radicals and the extent of oxidative stress is often quantified in plasma or cells by measuring the amounts of diagnostic molecules such as oxidatively modified lipids, proteins, antioxidants, and depolymerization of hyaluronic acid (Baker *et al.* 1989; Anderson & Theron 1990; Deeble *et al.* 1990; Sato *et al.* 1990; Jialal & Grundy 1991; Hawkins & Davies 1996; Uchida *et al.* 1998). We return below to the latter two measurements of oxidative stress, because they are of particular relevance to condition-dependent ornamentation (figure 1).

(a) *Toxication*

The activity of phase-I biotransformation enzymes often produces a reactive metabolite that the phase-II enzymes usually transform into an inactive water-soluble compound, which can be excreted. However, owing to, for example, exhaustion of biotransformation enzymes, consumption of their cosubstrates (for example glutathione), or the properties of the substrate and the active site of the enzyme (Kappus 1993), the metabolite may not be properly processed. In this process, called toxication (Gregus & Klaassen 1996), the metabolite is transformed into an even more reactive compound, which eventually generates free radicals (Burton & Ingold 1984; Kappus 1993; Gregus & Klaassen 1996; Parkinson 1996; Nebert *et al.* 1996).

In a number of studies, genetic polymorphisms in human biotransformation enzymes are correlated with an increased risk of toxicity and cancer owing to their generation of free radicals (reviewed by Daly *et al.* 1993; Rosvold *et al.* 1995; Nebert *et al.* 1996). In addition, a recent study on the Atlantic tomcod (*Microgadus tomcod*) indicates that a genetic adaptation in this fish species has reduced the biotransformation-induced toxication of dioxin-related compounds (Roy & Wirgin 1997). In highly polluted environments the frequency of various Ah receptor alleles has changed; this change in frequency has led to a downregulation of the Ah receptor pathway and elimination of the incidence of tumours that had previously prevailed in the population (Roy & Wirgin 1997).

(b) *Inflammatory response*

The cytotoxic effects of free radicals are exploited by phagocytes when obliterating pathogens in the inflammatory response (Klebanoff & Clark 1978). When pathogenic organisms enter the body they are first attacked by various phagocytes, such as macrophages and neutrophils: this is the so-called innate immune response. Phagocytes do not require pathogen-specific antibodies for the recognition process, because they also express non-specific receptors for various complement proteins that facilitate binding to the pathogen (Roitt *et al.* 1996). When the phagocytes have ingested or adhered to alien cells or parasites they are destroyed by the release of lysosomal enzymes, which catalyse the respiratory burst (Klebanoff & Clark 1978). This is a rapid reaction that gives rise to the formation of various free radicals and oxidants, such as hydrogen peroxide, which eventually decay to the highly obnoxious hydroxyl radical (Halliwell & Gutteridge 1985). The reactive products released by the phagocytes, intended to kill the pathogen, will also be harmful to other exposed cells (see, for example, Halliwell & Gutteridge 1985; Gruner *et al.* 1986; Anderson & Theron 1990; Ahmad 1995) and will thus contribute to oxidative stress.

Once activated, macrophages present antigens, bound to their MHC molecules, from ingested pathogens to T-cells (Roitt *et al.* 1996). MHC-mediated recognition is essential for the adaptive immune system to mount a specific and more effective response (shorter time lag, higher antibody titre and higher antibody affinity) on subsequent infections (Rajewsky 1996; Roitt *et al.* 1996). If pathogens escape the adaptive immune response, owing to the absence of critical MHC alleles, much of the immunological response will rest upon the less specific recognition and binding by the innate immune system's phagocytes.

Hence, individuals whose battery of MHC molecules fail to bind efficiently to the peptide fragments from the pathogen will produce a less efficient, low-affinity, antibody-mediated response (Roitt *et al.* 1996). A less specific defence may be costly in that it generates prolonged periods of sickness and extensive oxidative stress. The level of oxidative stress can therefore operate as a reliable measure of the genotype–environment interactions of the immune system.

4. ANTIOXIDANT DEFENCES

A series of antioxidant defence mechanisms have evolved to prevent or limit free-radical production and tissue damage. Superoxide dismutase (SOD), catalase and glutathione peroxidase (GPO) are endogenous enzymes that function as antioxidants inside cells. Extracellular antioxidants, such as vitamin C, carotenoids and vitamin E, are often of dietary origin (Maguire *et al.* 1989; Frei *et al.* 1992) and act by directly scavenging oxidants (Burton & Ingold 1984; Liebler 1993). Different types of antioxidants can act in a complementary or synergistic way to each other, so that the reaction of one antioxidant can spare or even regenerate others (Anderson & Theron 1990; Sato *et al.* 1990; Jialal & Grundy 1991; Olanow 1993). The dietary antioxidants are consumed during their antioxidant action (Maguire *et al.* 1989; Frei *et al.* 1992; Liebler 1993) and this makes their amounts in plasma and tissue potential measures of the level of oxidative stress (Anderson & Theron 1990; Sato *et al.* 1990; Jialal & Grundy 1991). Plasma and tissue levels of vitamin C, vitamin E, and carotenoids are reduced by 35–75% in birds and mammals during immune responses to infectious diseases (Ruff *et al.* 1974; Sykes 1979; Augustine & Ruff 1983; Hennet *et al.* 1992).

Experimentally increased concentrations of vitamin C, β-carotene and vitamin E reduce the negative effects of free radicals on various molecules and the immune response (Weitberg *et al.* 1985; Anderson & Theron 1990; Jialal & Grundy 1991; Hughes *et al.* 1997). Causal effects of antioxidant defence and oxidative stress on ageing have

recently been demonstrated in *Drosophila melanogaster*: flies from transgenic lines simultaneously overexpressing SOD and catalase exhibit not only a significant extension of lifespan but also improved metabolic rate and physical performance (Sohal *et al.* 1995).

The level of oxidative stress seems to be so intimately linked to health and fitness that it cannot be freely traded with reallocations of non-fitness-related currencies. Apart from the synthesis of the endogenous antioxidants and the reduction of glutathione, a cosubstrate to GPO (Gregus & Klaassen 1996), much of the cost of antioxidant defences is restricted to the depletion of extracellular dietary antioxidants. Accordingly, depletion of the deposits of such antioxidants is observed not only during immune responses but also after exhausting physical activities (Packer *et al.* 1994).

5. CONDITION-DEPENDENT SEXUAL SIGNALS

The hypothesis that sexual ornaments reveal the level of oxidative stress is theoretically appealing because any change that diminishes the level of oxidative stress would improve both the fitness and the expression of the ornament. Given that the degree of sexual ornamentation is limited primarily by an energy trade-off, as often suggested, one would expect male ornaments to be energetically costly to develop or maintain. Accordingly, female choice would promote a male strategy to squander energy, generated by mitochondrial metabolism, on characters that increase male mating success but do not contribute *per se* to survival, just to ensure the honesty of ornamental expression. The advantage of female preferences for male characters that instead reveal the status of the bearers' antioxidant defence systems and overall load of oxidative stress is that the honesty of the signal is manifested primarily by metabolic efficiency rather than by energy expenditure (see also Getty 1998). Females can thereby not only achieve healthy offspring but also produce attractive sons that honestly signal their genetic quality at low energetic expenditure.

To suppress the extent of oxidative stress without compromising mitochondrial respiration it seems essential to minimize the production of free radicals generated by other physiological processes, such as immune defence and detoxication. In these defence systems, different genotypes will interact differently with the environment (pathogens and xenobiotics); these interactions will affect the quantity of free radicals added to an individual's baseline (respiratory) level of oxidative stress (figure 1). A direct association between Ah receptor alleles and the resulting oxidative stress after administration of dioxin has been found in congenic mice (Alsharif *et al.* 1994; Hassoun & Stohs 1996). In chicken, MHC haplotypes associated with resistance to coccidiosis affect plasma levels of carotenoids after exposure to the disease (Uni *et al.* 1995).

Given that tolerance or resistance towards such environmental challenges can be inherited, female choice should promote the evolution of male ornaments that reliably reveal the status of the bearers' antioxidant defence systems or that are otherwise particularly sensitive to oxidative stress. Below we give examples of a wide array of sexual ornaments that may be particularly sensitive to oxidative damage.

(a) *The songbird's song and neurogenesis in the adult brain*

The vertebrate brain consumes a disproportionate amount of the body's oxygen (Coyle & Puttfarcken 1993) and several enzymes expressed in the brain produce free radicals (Olanow 1993). The brain also contains large amounts of polyunsaturated fatty acids, which are particularly vulnerable to free radicals (Coyle & Puttfarcken 1993). Accordingly, free radicals seem to contribute to several neurodegenerative disorders in humans, including Parkinson's disease and Alzheimer's disease (Coyle & Puttfarcken 1993; Olanow 1993).

In the brains of adult songbirds, neurons continue to be produced and integrated into the high vocal centre (HVC), which is involved in the control of learned vocalization (Alvarez-Buylla 1992; DeVoogd *et al.* 1993). In the genus *Acrocephalus* a large song repertoire seems to be important for female mate choice, like secondary ornaments in other bird species (Catchpole 1986). Female great reed warblers (*Acrocephalus arundinaceus*) rarely seek extrapair copulations (Hasselquist *et al.* 1995) but when they do so they prefer to mate with a neighbouring male with a song repertoire larger than that of the pair male (Hasselquist *et al.* 1996). Relative postfledging survival, in terms of offspring returning from the overwintering areas in Africa to their natal area in Sweden, is positively correlated with the genetic fathers' song repertoire size even when the effects of male age and paternal care are controlled for (Hasselquist *et al.* 1996).

The size of a bird's song repertoire correlates with the volume of the HVC (Nottebohm 1981; Kroodsma & Canady 1985; DeVoogd *et al.* 1993) and with developmental stress (Nowicki *et al.* 1998). There is a seasonal plasticity in both the size of the HVC and the rate at which new neurons are incorporated into the HVC (Nottebohm 1981; Alvarez-Buylla 1992). In a number of bird species, the volume of the HVC is up to 70% larger in breeding than in non-breeding individuals (Nottebohm 1981; Brenowitz *et al.* 1991).

Recent data indicate that free radicals have negative effects on neurogenesis in the developing brain (Saito *et al.* 1997). Neurogenesis also occurs in the hippocampus of adult rodents and birds (Altman & Das 1965; Barnea & Nottebohm 1994) and there is an increasing number of experimental studies demonstrating the damaging effects of free radicals on the function of the hippocampus (Sugaya *et al.* 1996; Behl *et al.* 1997; McIntosh *et al.* 1998; Vornov *et al.* 1998), such as loss of neurons and impairments in spatial learning and cognition (McEwen & Sapolsky 1995).

The hippocampus is essential for spatial memory and cognition (Sherry *et al.* 1992; Bingman & Jones 1994) and its function may therefore be an important fitness component, especially in food-storing and migratory animals. In analogy to the HVC, both the volume and number of neurons in the hippocampus vary seasonally in accordance with food-storing behaviour (Barnea & Nottebohm 1994; Smulders *et al.* 1995). In addition, food-storing bird species have relatively larger volumes of hippocampus, and more neurons as well, than non-storing species (Healy & Krebs 1993), and in songbirds the relative size of the hippocampus correlates with migratory habits (Healy *et al.* 1996).

Recent experimental work reveals that mice show dose-dependent reductions in spatial learning in response to intestinal parasite infections without any pathological responses (Kavaliers *et al.* 1995). Age-induced impairment in spatial learning is directly associated with oxidative stress in the hippocampus of rats (Sugaya *et al.* 1996); administration of antioxidants decreases free-radical oxidation in the brain and improves spatial memory and cognition in elderly rodents (Carney *et al.* 1991).

(b) *The cock's comb*

Comb size in male red jungle fowl (*Gallus gallus*) correlates positively with female choice, condition and survival (Zuk *et al.* 1990). The rooster's comb has by far the highest concentration of hyaluronic acid (HA) known (Laurent & Fraser 1992). HA is a straight-chain polysaccharide forming a highly viscous solution that affects the balancing homeostasis of water and plasma proteins in the intercellular matrix (Laurent & Fraser 1992). The depolymerization of HA is promoted by free radicals, in particular $OH^{\cdot}$, which cause strand breakage and loss of viscosity of HA molecules (Baker *et al.* 1989; Hawkins & Davies 1996) at a dose-dependent rate (Deeble *et al.* 1990); these reactions are prevented by administration of antioxidants (Kvam *et al.* 1993; Saari *et al.* 1993). Activated phagocytes diminish the viscosity of HA in synovial fluid and tissue through their generation of free radicals (Grootveld *et al.* 1991; Saari *et al.* 1993); experimental data show that the size and shape of the rooster's comb rapidly deteriorate in response to infections (Zuk *et al.* 1990).

(c) *Avian plumes and spurs*

Male plumes in many bird species are perhaps the most extravagant sexual ornaments in animals (Andersson 1994; Møller 1994). Avian feathers and spurs consist of keratin polypeptides synthesized by epidermal keratinocytes (Haake & Sawyer 1986). Experiments *in vitro* have demonstrated that hydrogen peroxides inhibit keratinocyte proliferation (O'Toole *et al.* 1996) and that vitamin C promotes the proliferation of mammalian keratinocytes (Saika *et al.* 1991). Overall, cutaneous ageing, such as decreased turnover of epidermal cells, loss of mature collagen and keratinocytes, and decreases in hair and nail growth, is associated with increased generation of free radicals (Cerimele *et al.* 1990). Reduced collagen synthesis, which is frequently associated with aged skin and scurvy, can be reversed by treatment with vitamin C (Hata *et al.* 1988).

In pheasants (*Phasianus colchicus*) male MHC genotype is significantly associated with survival and with the length of the tarsial spurs (von Schantz *et al.* 1996); experimental data show that spur length affects female choice (von Schantz *et al.* 1989). Parasitic infections have a negative effect on the length of ornamental tail feathers in several bird species (Zuk *et al.* 1990; Møller 1994; Andersson 1994). In particular, parasite load and viral infections during moulting have marked negative effects on both the length of wing feathers and carotenoid pigmentation of ornamental feathers (Thompson *et al.* 1997).

(d) *Carotenoids*

In fishes and birds, males often display sexual signals through reddish skin or plumage coloration. This pigmentation usually consists of carotenoids; females prefer males with more reddish coloration (Endler 1983; Hill 1991; Milinski & Bakker 1990). In guppies (*Poecilia reticulata*) and sticklebacks (*Gasterosteus aculeatus*) redder males are in better condition (Milinski & Bakker 1990; Nicoletto 1993) and less parasitized (Milinski & Bakker 1990) and the red coloration is particularly sensitive to parasitic infections (Houde & Torio 1992). Carotenoids seem to be essential to juvenile Atlantic salmon (*Salmo salar*) during their first feeding period (Christiansen *et al.* 1995). Experimental sib-group analyses on Atlantic salmon, controlling for the effects of food quality, reveal significant genetic effects on the variation in tissue content of carotenoids (Torrissen & Naevdal 1988).

The physiological mechanisms that relate male condition to carotenoid pigmentation and to what extent this correlation is governed by intrinsic or extrinsic factors are controversial issues among evolutionary biologists (Thompson *et al.* 1997). The main hypothesis to explain the proximate basis for the condition-dependence of carotenoids has been that males with more carotenoid pigmentation are better foragers and therefore more viable (Endler 1980; Hill 1992) but this ignores the carotenoids' function as antioxidants (Burton & Ingold 1984; Lozano 1994) and their depletion in response to oxidative stress (Andersson & Theron 1990; Frei *et al.* 1992).

6. CONCLUSION

An organism's ability to combat pathogens is highly dependent on the immune system's ability to find the right target and at the same time avoid damage to itself (Råberg *et al.* 1998). On the other hand, the pathogens' ability to survive and proliferate depends on their ability to escape recognition and to exploit the immune system's regulatory mechanisms. In the course of evolution, selection has moulded several different strategies in the ongoing dynamic battle between pathogens and their hosts and between plants and their herbivores. Genetic resistance does not necessarily result in parasite-free or completely detoxicated individuals, but can be manifested as high tolerance to common parasites (Skarstein & Folstad 1996; Zinkernagel 1996) or foreign compounds (Gregus & Klaassen 1996; Kalow 1997; Roy & Wirgin 1997).

The defence systems governed by the polymorphic gene complexes discussed here generate free radicals to a degree that is modulated by tolerance and resistance to exposure. Even if the genetic differences are small, tolerant individuals probably generate fewer reactive metabolites and free radicals than individuals with less genetic resistance or tolerance. For example, by selecting the male with the largest song repertoire among several other seemingly healthy males, the female warbler may maximize the chances that she will pass on alleles to her offspring that improve fitness-related functions, such as spatial memory, that are negatively affected by elevated levels of oxidative stress.

We point to several cases where male ornaments favoured by female choice seem to be particularly susceptible to oxidative stress. It is certain that the free-radical-generating defence systems reviewed here are not the only factors affecting the condition-dependent expression of

secondary ornaments. For example, glucocorticoids and sex hormones can interact with the biotransformation (Prough *et al.* 1996) and immune (Folstad & Karter 1992; Besedovsky & del Rey 1996; Råberg *et al.* 1998) systems, and thereby affect the production of free radicals as well as directly affecting the expression of ornaments (Johns 1964; Marler *et al.* 1988). In the immunocompetence-handicap hypothesis, Folstad & Karter (1992) suggested that steroid hormones function as mediators of honest sexual signalling, because high steroid hormone levels confer costs through suppressive effects on the immune system. Such a trade-off may be modulated by oxidative stress: glucocorticoids, testosterone and progesterone are known to impair the enzymic antioxidant defences or directly induce oxidative stress in various tissues (see, for example, Behl *et al.* 1997; Chainy *et al.* 1997; Zhu *et al.* 1997; McIntosh *et al.* 1998). Hence, in addition to the immune and biotransformation systems there are other physiological mechanisms, controlled by less variable genes, which add to the overall load of reactive metabolites. Given that the allelic variants of the immune and biotransformation multilocus systems (table 1), in dynamic interactions (Kirzhner *et al.* 1996) with pathogens and xenobiotics, additively generate oxidative stress to a level that relates to the individual's overall genetic resistance or tolerance to exposure (figure 1), then female mate choice can enhance offspring fitness (Eshel & Hamilton 1984; Hamilton *et al.* 1990).

In many cases it has been shown that ornaments disclose the bearer's health and condition (see, for example, von Schantz *et al.* 1989; Milinski & Bakker 1990; Zuk *et al.* 1990; Hill 1991; Houde & Torio 1992; Nicoletto 1993; Andersson 1994; Møller 1994; Thompson *et al.* 1997), and it is now time to experimentally study the physiological mechanisms affecting the expression of these ornaments. To separate the effects of oxidative stress on the expression of sexual ornaments from the effects of energy reallocation calls for experiments at which the level of oxidative stress can be manipulated while controlling for other means of physiological stress, such as workload or exposure to pathogens. Increased oxygen pressure (hyperoxia) and exposure to hydrogen peroxide below pathological levels have previously been used to induce oxidative stress both *in vivo* and *in vitro* (Orr & Sohal 1992; Auerbach & Segal 1997). Such experiments and experimental infections of pathogens in combination with treatment of various antioxidants may shed light on the causal links between sexual ornaments and individual fitness.

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