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Carotenoid ornaments and the spandrels of physiology: a critique of theory to explain condition dependency

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ABSTRACT

Even as numerous studies have documented that the red and yellow coloration resulting from the deposition of carotenoids serves as an honest signal of condition, the evolution of condition dependency is contentious. The resource trade-off hypothesis proposes that condition-dependent honest signalling relies on a trade-off of resources between ornamental display and body maintenance. By this model, condition dependency can evolve through selection for a re-allocation of resources to promote ornament expression. By contrast, the index hypothesis proposes that selection focuses mate choice on carotenoid coloration that is inherently condition dependent because production of such coloration is inexorably tied to vital cellular processes. These hypotheses for the origins of condition dependency make strongly contrasting and testable predictions about ornamental traits. To assess these two models, we review the mechanisms of production of carotenoids, patterns of condition dependency involving different classes of carotenoids, and patterns of behavioural responses to carotenoid coloration. We review evidence that traits can be condition dependent without the influence of sexual selection and that novel traits can show condition-dependent expression as soon as they appear in a population, without the possibility of sexual selection. We conclude by highlighting new opportunities for studying condition-dependent signalling made possible by genetic manipulation and expression of ornamental traits in synthetic biological systems.

Key words: ornamentation, animal coloration, index signal, resource trade-off hypothesis, honest signalling, ketocarotenoids.

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I. INTRODUCTION

A central question in evolutionary biology is, how do condition-dependent signals of quality evolve and remain reliable through time (Hamilton & Zuk, 1982; Kodric-Brown & Brown, 1984; Andersson, 1994; Smith & Harper, 2003)? Nearly all models emerging from sexual selection theory hold that honest signals of individual quality evolve in response to adaptive mate choice by females (Zahavi, 1975; Grafen, 1990; Kuijper, Pen & Weissing, 2012; Hill & Johnson, 2013). Gould and Lewontin (1979) famously highlighted the pitfalls of invoking adaptive stories for the origin of a given trait and emphasised that traits might be a consequence of historical contingency, phyletic inertia, and genetic drift rather than adaptive evolution. They used as an analogy the spandrels of the San Marco Cathedral – where curved spaces between adjacent arches appear to have been created for the purpose of framing painting masterpieces (Fig. 1). In truth, the spandrels are a simple consequence of the arched supports needed to hold up the expansive ceiling of the church; once such odd nooks existed, they were made beautiful with art. Here we extend the cautionary note about adaptive storytelling of Gould & Lewontin (1979) to explanations for the evolution of condition-dependent carotenoid coloration.

Ornaments used in mate assessment are interesting because their expression is variable among individuals in a population despite being subject to strong directional selection (Taylor & Williams, 1982; Hamilton & Zuk, 1982; Kirkpatrick & Ryan, 1991). Expression of such ornaments is often correlated with measures of individual condition or quality (Kodric-Brown & Brown, 1984; Hill, 1991; Weaver, Koch & Hill, 2017). Moreover, such traits are often used in mate assessment because they convey information about the quality or condition of prospective mates (Kodric-Brown, 1989; Andersson, 1994; Hill, 2015). According to the indicator trait hypothesis, only individuals with superior genotypes or phenotypes can meet the challenges of the production or maintenance of ornamentation, and a choosing individual gains important information about the genotype or phenotype of a prospective mate by assessing such a condition-dependent ornament (Cotton, Small & Pomiankowski, 2006; Johnstone, Rands & Evans, 2009; Hill, 2011). Condition-dependent signalling has become a cornerstone of sexual selection theory and a foundational concept in evolutionary biology, but the origins of condition dependency remain a contentious topic in evolutionary biology (Maynard Smith & Harper, 1995; Houle & Kondrashov, 2002; Bonduriansky, 2007; Higham, 2014; Bath *et al*., 2023).

II. HONESTY ARISING FROM TRADE-OFFS *VERSUS* INDEX SIGNALS

Condition is traditionally defined in terms of the quantity of metabolic resources possessed by an individual, with high-condition individuals having a larger pool of resources and hence greater capacity for production of ornaments

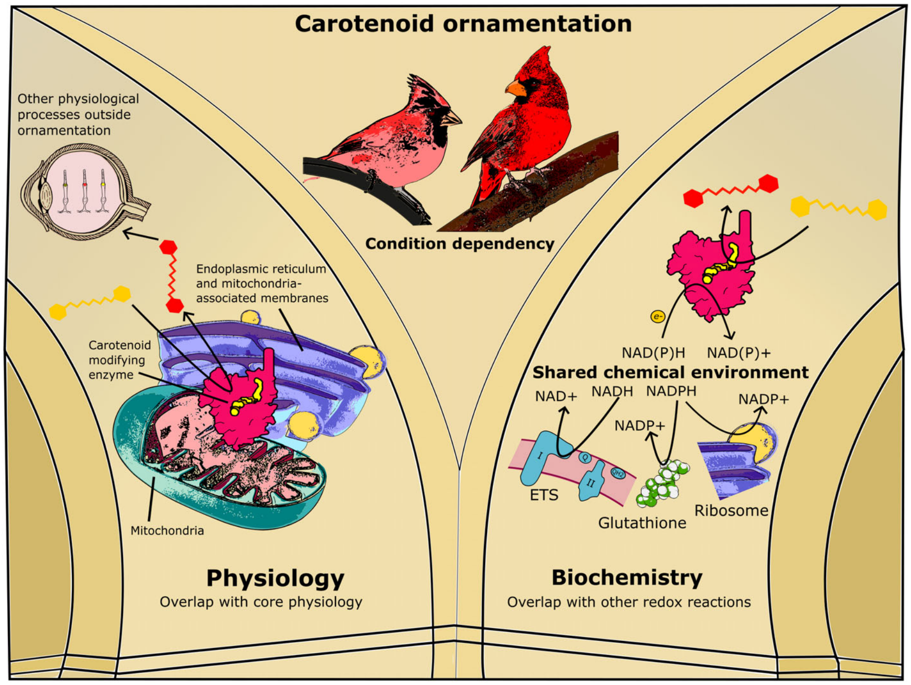

Fig. 1. The spandrel of condition-dependent carotenoid ornamentation created by the arched supports of physiology and biochemistry. The condition dependence of carotenoid-based ornaments (the spandrel) does not exist because carotenoid condition dependence is a means to an end (i.e. for the purpose of sexual selection), but rather because it is the end result of inherent physiological processes (i.e. the components of cellular function depicted on the archways). ETS, electron transport system.

(Zeh & Zeh, 1988; Wilkinson & Taper, 1999; Morehouse, 2014; Koch & Hill, 2018). From this definition of condition arose the hypothesis that sexual selection for greater expression of display traits leads to the evolution of mechanisms for diversion of resources to ornamental trait production (Rowe & Houle, 1996). According to this theory, optimal allocation is determined by the relative costs and benefits of trade-offs, such that individuals in high condition can afford to invest more in ornamental traits than individuals in low condition (Getty, 1998, 2006; Höglund & Sheldon, 1998; Morehouse, 2014). In support of the idea that condition-dependent honest signalling evolves under the influence of sexual selection, it is sometimes observed that traits under sexual selection show heightened condition dependence compared to traits that are not subject to sexual selection (Cotton *et al.*, 2006; Bonduriansky, 2007). In an experimental study, however, Bath *et al.*, (2023) found no support for the hypothesis that the condition dependency of traits increases under sexual selection.

The index hypothesis challenges the hypothesis that condition dependency evolves under the influence of sexual selection. By the index hypothesis, condition dependency is an inherent property of some traits; the benefits of choosing the best individuals as mates then favour the evolution of behavioural traits that cause choosing individuals to be attracted to ornaments that signal individual condition (Johnstone, 1995; Maynard Smith & Harper, 1995). In other words, the index hypothesis proposes that sexual selection leads to evolution of attraction to traits that *a priori* provide information about condition. Several examples of how traits can exist as inherently honest signals have been suggested (Maynard Smith & Harper, 1995), but one of the most important hypotheses is the shared pathway hypothesis, which proposes that traits can be condition dependent if ornament production is inexorably linked to core cellular pathways required for vital life processes (Hill, 2011; Warren *et al.*, 2013; Biernaskie, Grafen & Perry, 2014). Under this framework, condition-dependent ornaments need not be promoted by sexual selection; instead, some traits are inherently tied to individual condition because they are tied to fundamental life processes. Moreover, the shared pathway hypothesis challenges the idea that the condition of an individual is determined by the metabolic resources it carries, because condition arises from the functionality of vital cellular processes, not resource trade-offs (Hill, 2011). When such index traits are easily assessed in a phenotype, they are co-opted for mate assessment through sexual selection. Just as the spandrels in the San Marco Cathedral exist as a necessity of the architecture of a large church, some biochemical traits in animals exist as honest signals because of necessities in the cellular architecture of animal systems.

III. PREDICTIONS OF THE RESOURCE TRADE-OFF AND INDEX HYPOTHESES

The resource trade-off and index hypotheses for the origin of condition dependency make contrasting predictions (Biernaskie *et al.*, 2014; Weaver *et al.*, 2017). The resource trade-off model proposes that condition dependency requires resource costs for ornamentation. One could imagine an ornament that entails resource trade-offs arising *de novo* in a population, but we are aware of no such examples. Rather, models for the origin of condition-dependent social signalling invariably propose that such signalling arises in response to sexual selection for ornament expression (Rowe & Houle, 1996; Bath *et al.*, 2023). Key testable predictions follow (Tomkins *et al.*, 2004). First there should be evidence of resource trade-offs in the production of ornamental traits, and traits with higher resource costs should capture more condition-dependent variation (i.e. be more reliable signals of condition). Second, because condition dependency evolves over generations through selection for mechanisms for the re-allocation of resources, the resource trade-off hypothesis predicts that ornaments will become more condition dependent over evolutionary time. Third, because condition-dependent signalling emerges as a consequence of sexual selection on a trait, traits that appear ornamental but that occur in taxa with no means to perceive the display, and hence with no sexual selection for the trait, should show little condition dependency.

By contrast, the index hypothesis does not invoke resource trade-offs as the basis for honest signalling, nor does it require that a trait be the target of sexual selection to be a strong signal of individual condition (Hill, 2011). The index hypothesis proposes that some traits exist as inherently honest signals (Maynard Smith & Harper, 1995). Thus, key predictions of the index hypothesis are, first, that experiments will not confirm a fitness cost of resource allocation to ornament expression. Second, the index hypothesis predicts that whether or not an ornament is subject to sexual selection, expression of the trait will be associated with individual condition; in other words, traits do not become more strongly linked to condition dependence over evolutionary time. And finally, under the shared pathway hypothesis, as a specific form of the index hypothesis, there should be mechanisms that would make an ornament a reliable signal of condition without the need for resource-based trade-offs.

IV. CAROTENOID COLORATION AS A MODEL CONDITION-DEPENDENT TRAIT

In this review we assess predictions of two competing hypotheses for the origin of condition-dependent expression of carotenoid coloration, a textbook example of a condition-dependent trait (Svensson & Wong, 2011; Dugatkin, 2020). Carotenoids produce bright red and yellow coloration in the skin, feathers, and scales of many species of animals, and such colour displays have often been shown to be condition dependent because of biochemical properties of the pigments themselves and because of the hypothesized physiological requirements of the host cellular environment for their accumulation.

Both the resource trade-off hypothesis and the index hypothesis have been proposed to explain carotenoid coloration in animals (Birkhead *et al.*, 2006; Alonso-Alvarez *et al.*, 2008; Weaver *et al.*, 2017). The resource trade-off hypothesis proposes that allocation of carotenoids to ornamentation evolves to be more condition dependent under sexual selection (Boughman, 2007; Friesen *et al.*, 2017). By contrast, the index hypothesis posits that carotenoid coloration is inherently condition dependent and that such ornaments are co-opted as signals of condition in social signalling (Hill, 2014; Weaver *et al.*, 2017). The hue or chroma of ornamental carotenoid coloration has been linked to aspects of individual condition, including body condition, diet, immunocompetence, parasite load, oxidative stress, and physical performance in numerous studies of birds and fish [reviewed in Kodric-Brown (1989), Hill (2002), Svensson & Wong (2011) and Sefc, Brown & Clotfelter (2014)], as well as in studies of other classes of vertebrates (Fitze *et al.*, 2009; Badiane *et al.*, 2022) and arthropods (e.g. Davenport *et al.*, 2004; Hsiung *et al.*, 2017; Weaver *et al.*, 2018*c*). We present an overview of the mechanisms of production of carotenoids, patterns of condition dependency involving different classes of carotenoids, and the patterns of behavioural responses to carotenoid coloration to assess whether observations support the idea that the condition dependency of carotenoid ornaments evolves under the influence of sexual selection or that carotenoid coloration is an inherently condition-dependent trait.

V. SOURCES OF CAROTENOIDS USED IN ORNAMENTAL COLORATION

Carotenoids are the primary source of red and yellow coloration birds (Mcgraw, 2006), turtles (Steffen *et al.*, 2015) and crustaceans (Weaver, Cobine & Hill, 2018*a*) and play important roles in yellow and red coloration in fish (Luo *et al.*, 2021) and reptiles (Olsson, Stuart-Fox & Ballen, 2013). In vertebrates and, with very few exceptions (Altincicek, Kovacs & Gerardo, 2012), in invertebrates, carotenoids cannot be synthesised *de novo* from basic precursor molecules; they must be ingested (Goodwin, 1984; Weaver *et al.*, 2018*a*). Carotenoids are used as ornamental colorants through one of three biochemically distinct processes: (*i*) ingested, dietary pigments are used without biochemical modification; (*ii*) dietary pigments are biochemically modified into ε,ε-carotenoids *via* a dehydrogenase reaction; or (*iii*) dietary pigments are biochemically modified into keto-carotenoids *via* a ketolation reaction using the products of a dehydrogenation reaction as precursors, which often involves a pigmentary colour change, usually yellow to red (Mcgraw, 2006; Hill & Johnson, 2012; Toomey *et al.*, 2022) (Fig. 2). Decades of work on the pigmentary basis for yellow and red coloration in birds has revealed that all three means of displaying carotenoid coloration are common in Aves (Mcgraw, 2006; Friedman, McGraw & Omland, 2014; Ligon *et al.*, 2016) as well as in closely related but extinct taxa (Davis & Clarke, 2022) and that all three sources of coloration are used at least

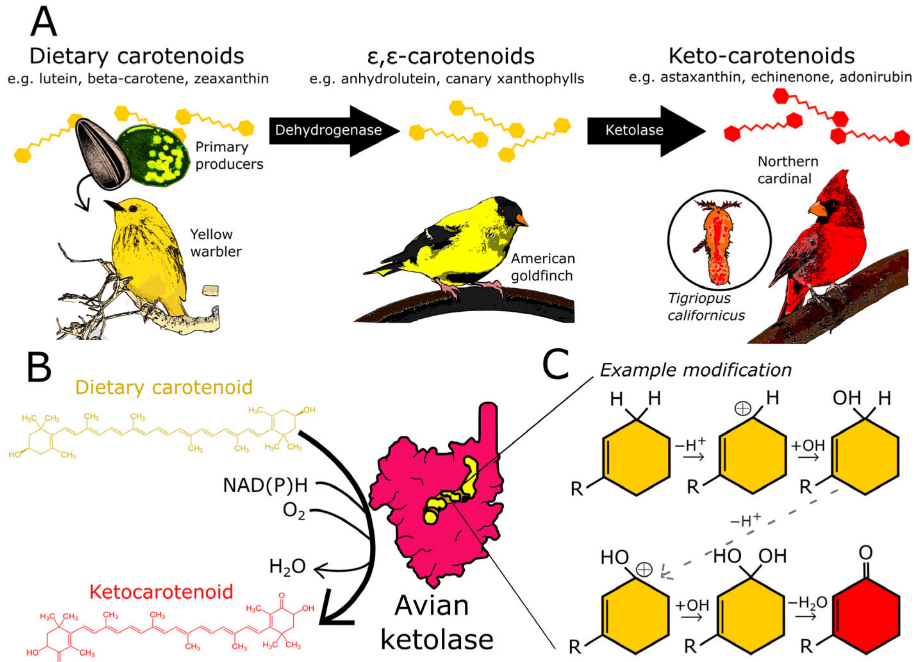

Fig. 2. An example of the diversity of carotenoid biochemistry and modification. (A) Examples of dietary, ε,ε-carotenoids, and ketolated carotenoids employed in the ornaments of various species. (B) Example of a ketolation reaction driven by an electron donor molecule, NADPH, and catalysed by a ketolase enzyme. (C) Example of a reaction mechanism of a ketolation reaction that occurs within a modification enzyme's active site. This particular reaction shows the steps necessary to add a ketone group during the process of C4-oxygenation.

occasionally in diverse taxa of vertebrates and invertebrates (Goodwin, 1984).

The biochemical process of carotenoid modification is not unique to groups that consume carotenoid resources. Carotenoid modification is also found in the primary producers of carotenoids like algae, bacteria, and flowering plants suggesting that the metabolic pathways involved in carotenoid processing are ancient and widespread across the evolutionary tree of life (Lafountain, Prum & Frank, 2015; Toews, Hofmeister & Taylor, 2017; Sandmann, 2021). Carotenoids used for coloration are often modified by enzymes that perform hydroxylations, dehydrogenations, or oxygenations to the terminal ionone rings of C-40 carotenoids (Lafountain *et al*., 2015, Chi *et al*., 2015; Prado-Cabrero, Saefurahman & Nolan, 2020). These reduction–oxidation (redox) reactions transfer electrons from mobile carriers such as NADH to the benzene rings of the carotenoids resulting in the addition of oxygen moieties and a change in the wavelength of light reflected by the molecule's chromophore (colour-producing component); often from yellow to red wavelengths (Fig. 2).

VI. WEAK EVIDENCE FOR RESOURCE TRADE-OFFS RELATED TO CAROTENOID COLORATION

Because carotenoids must be acquired in the diet (Goodwin, 1984) and could therefore represent a finite resource, the original discussions of honest signalling involving carotenoids focused simply on potential scarcity of carotenoids in the diets of animals, rather than trade-offs (Endler, 1980; Hill, 1992; Hudon, 1994). Under this carotenoid scarcity hypothesis, animals are challenged to accrue sufficient carotenoids for full ornament display, and only vigorous and robust individuals can meet this foraging challenge (Hill, 2002). Subsequently, it was proposed that carotenoids serve key roles in immune function and as free radical scavengers such that use of scarce dietary pigments had to be traded off between ornamentation and body maintenance (Lozano, 1994; von Schantz *et al*., 1999). This proposed trade-off between allocation of carotenoids to ornamentation *versus* to body maintenance became the dominant hypothesis for how carotenoid coloration was maintained as an honest signal of quality (Hill, 1999; Moller *et al*., 2000; Alonso-Alvarez *et al*., 2008). The need to trade off use of carotenoids for ornamentation *versus* body maintenance is a key assumption of the resource trade-off hypothesis as it pertains to carotenoid ornamentation (Rowe & Houle, 1996; Tomkins *et al*., 2004; Birkhead *et al*., 2006).

There is little direct evidence for trade-offs between use of carotenoids for ornamentation *versus* use of carotenoids for body maintenance (Fitze *et al*., 2007; Costantini & Møller, 2008; Koch & Hill, 2018). Numerous studies have attempted to test the hypothesis by creating an immune or oxidative challenge and then looking for a predicted loss of coloration as carotenoids are re-allocated from ornamentation to maintenance [reviewed in Svensson & Wong (2011) and Koch & Hill (2018)]. The unyielding complication for studies that attempt to assess trade-offs with such experiments, however, is that activation of the immune system or induction of oxidative stress can reduce carotenoid coloration by affecting the mechanisms of their uptake, transport, or metabolism rather than through re-allocation of carotenoid molecules *per se* (Weaver *et al*., 2017; Koch & Hill, 2018). In the only test in which the potential for allocation of carotenoids to ornamentation or body maintenance was controlled through genetic manipulation, carotenoid allocation had no effect on body function. In these experiments, Koch *et al*. (2018, 2019) compared the level of oxidative stress and immunocompentence of common canaries (*Serinus canaria*) with (*i*) species-typical genes that enable absorption of carotenoids from food and production of yellow feathers, (*ii*) a genetic mutation that stopped the uptake of carotenoids, making the bodies of these birds free of carotenoids, or (*iii*) genes that enabled the uptake of carotenoids but also with a genetic mutation that stopped the allocation of carotenoids to feather ornaments, giving the birds white plumage and eliminating all costs of allocation to ornamentation. There was no effect of allocation of carotenoids to ornamentation on either the immunocompetence or oxidative state of birds. As a matter of fact, birds with no circulating carotenoids – i.e. no 'carotenoid resources' at all – functioned as well as birds with high circulating levels of carotenoids. The canary studies were only one attempt to test the resource trade-off hypothesis directly, and the findings of these studies need to be corroborated with additional data from other systems. Nevertheless, failure to identify evidence of trade-offs in such extreme avian phenotypes challenges the hypothesis that allocation of carotenoid resources is the basis for honest signalling *via* carotenoid coloration (Weaver *et al*., 2017; Koch *et al*., 2018, 2019).

Under the index hypothesis, the condition dependency of carotenoid coloration does not arise because carotenoids represent a pool of resources. Rather, carotenoid coloration is an honest signal of individual quality because the production of carotenoid ornamentation – which often includes redox-dependent modification of dietary carotenoids – is tied to the vital cellular processes that underlie high individual function (Fig. 1) (Hill, 2011; Weaver *et al*., 2017). By this idea, there is simply no means to produce the ornament without a highly functional cellular system and there is no prediction of carotenoids as a limited resource or for trade-offs in the use of carotenoids.

VII. INHERENT LINKS TO CELLULAR FUNCTION

Numerous studies have documented an association between carotenoid coloration and some aspect of individual condition [reviewed in Cotton, Fowler & Pomiankowski (2004), Hill (2006*a*) and Svensson & Wong (2011)]. To assess the strength of the association between carotenoid coloration and condition, Weaver *et al.* (2018*b*) conducted a meta-analysis assessing the outcomes of studies of carotenoid-based plumage ornaments and condition in birds. Unlike previous reviews and summaries, however, in this meta-analysis the authors separately assessed the strength of condition-dependent signalling for the two distinct forms of ornamental coloration (Fig. 2A). They found that ornaments produced using carotenoids that were modified from dietary pigments – either into yellow ε,ε-carotenoids or into red keto-carotenoids – had stronger relationships with measures of individual quality than ornaments produced using dietary carotenoids. Ornaments produced with dietary pigments did also tend to serve as signals of individual condition; the associations were simply not as strong as in modified carotenoids (Weaver *et al.*, 2018*b*).

In light of the observation that ornaments produced *via* modified pigments are more reliable signals of condition than ornaments produced with unmodified dietary pigments, the patterns revealed in comparative studies assessing the evolution of different forms of carotenoid pigmentation were interesting. In these comparative studies, there was evidence for numerous evolutionary transitions in the coloration of feathers and the types of carotenoids used as pigments (Andersson, Prager & Johansson, 2007; Prager & Andersson, 2010; Prum *et al.*, 2012; Friedman *et al.*, 2014; Ligon *et al.*, 2016). However, not all transitions were equally likely; evolutionary transitions from dietary carotenoids to modified carotenoids (both red and yellow) were almost never reversed (Friedman *et al.*, 2014) while transitions between yellow ε,ε-carotenoids and red keto-carotenoids were common in both directions (Prager & Andersson, 2010; Ligon *et al.*, 2016). The lack of transitions from modified to unmodified pigmentation suggests that the information content of dietary *versus* modified pigments is fundamentally different such that transitions from more effective to less-effective signals of condition simply do not occur. On the other hand, the frequent transitions between colour displays based on modified yellow ε,ε-carotenoids *versus* modified red keto-carotenoids suggest that these two forms of ornamentation are equally effective as signals of condition and hence interchangeable (Hill & Johnson, 2012; Hill, 2022). The enzymatic pathways that enable modification of carotenoids for colour displays appear to be the product of carotenoid modification for vision (Toomey *et al.*, 2015; Lopes *et al.*, 2016; Twyman *et al.*, 2016). The implication is that the mechanisms for carotenoid modification existed for reasons other than production of ornamental colour and were co-opted for external display (Twyman *et al.*, 2016).

A potential explanation for why modified carotenoid pigmentation is more tightly linked to individual condition than dietary pigmentation concerns the reactions required to produce modified carotenoids. Hill (2011) proposed that modified carotenoid pigments are honest signals of individual condition because the production of such pigments involves pathways that are closely linked to vital cellular processes (Fig. 1). This hypothesis was subsequently refined to propose that production of modified carotenoid pigments involves metabolic conversions that are affected by mitochondrial function (Hill, 2014; Hill *et al.*, 2019). The original proposal was vague as to how, specifically, altered mitochondrial function might affect production of modified carotenoid pigments, and hence how coloration might be tied to aerobic respiration (but see Johnson & Hill, 2013; Hill *et al.*, 2019). Recent discoveries help to clarify possible mechanisms.

A key discovery was that the production of ketolated carotenoids for ornamental red coloration is accomplished not solely by the enzyme cytochrome P450 2J19 (CYP2J19) as had been proposed (Lopes *et al.*, 2016; Mundy *et al.*, 2016). Rather, in at least fish, turtles, and birds, the conversion of yellow dietary pigments to red ketocarotenoids requires two enzymes: a dehydrogenase (3-hydroxybutyrate dehydrogenase 1-like, BDH1L, in birds) and a ketolase (CYP2J19 in birds; Toomey *et al.*, 2022; Fig. 2). The conversion of lutein, zeaxanthin, β-cryptoxanthin, or β-carotene to a yellow ε,ε-carotenoid by BDH1L is a necessary step in the production of a red ketolated carotenoid *via* CYP2J19. In crustaceans with carotenoid-based coloration, there may be only a single carotenoid-modifying enzyme; however, this enzyme may be multifunctional and capable of performing multiple types of reactions (Prado-Cabrero *et al.*, 2020; Weaver *et al.*, 2020). Many carotenoid-modifying enzymes discovered in vertebrates and invertebrates so far belong to superfamilies of oxygenases and dehydrogenases. These enzymes share similarities in the reactions they perform, and this biochemistry provides the requisite link to vital cellular processes in the mitochondria and mitochondrial-associated membranes of the endoplasmic reticulum (Fig. 1).

Carotenoid modifications involve the addition, subtraction, or swapping of functional groups on the benzene rings of the molecule (Fig. 2C). These changes ultimately tune the colour produced by the carotenoid through lengthening or shortening the chain of double bonds that run along the carbon backbone of the pigment. Carotenoid-modifying enzymes perform oxidation–reduction (redox) reactions to alter the functional groups on the carotenoid molecule. Enzymes in this class of compounds cycle electrons from an energy-rich donor molecule like NADH or NADPH to complete their reactions, producing NAD+ or NADP+ as a by-product (Figs 1 and 2B) (Nakahara *et al.*, 1993; Guengerich, 2018). Once depleted, NAD+ and NADP+ may be recharged with electrons and thus continue to drive redox reactions in a cyclical process. Dependence on redox cycles potentially ties the process of modifying carotenoids to the mitochondria and mitochondria-associated membranes, which are dependent on their own set of redox reactions to perform a variety of functions (Johnson & Hill, 2013; Hill *et al.*, 2019). Mitochondrial function has been shown to impact endoplasmic reticulum (ER) function through feedback loops of changes in the redox state of the mitochondria and the cytosol (Gansemer *et al.*, 2020). The ratios of electron donors like NADH and NADPH (and their oxidised forms) or redox products are in flux both in the mitochondria and the mitochondria-associated membranes of the ER (Fig. 1) and it is possible that conditions in one compartment may affect those in another through the shuttling of intermediate molecules across membrane barriers (Lan *et al.*, 2019; Li *et al.*, 2019).

The fluctuations in the ratios of redox partners and products in the cell, both within the mitochondria and at the mitochondria–ER boundary, constitute a shared redox environment (Fig. 1). Any enzymes within this changing electrochemical environment that utilise redox reactions are subject to changes in their electrochemical potential to perform those reactions (Titov *et al.*, 2016; Corkey & Deeney, 2020). By extension, any pathways dependent on energetic products from this environment should also be affected by changes in the rate of vital redox reactions, including downstream pathways that produce a variety of ornaments. Indeed, carotenoid modifications may be intertwined with energetic processes within the shared redox environment. Carotenoid-modifying enzymes are predicted to perform their redox reactions within the shared electrochemical environment of mitochondria or mitochondria-associated membranes of the ER (Johnson & Hill, 2013; Hill *et al.*, 2019). Therefore, the rate at which an organism can produce ketolated carotenoids could be linked to the metabolically determined redox environment of its relevant cells. Any alterations to bioenergetic metabolism may alter the redox environment for all associated redox-dependent reactions, including carotenoid enzymes, thus allowing carotenoid-based coloration to serve as a biochemical index of an individual's core cellular physiology (Powers & Hill, 2021).

This possibility is intriguing given evidence that modified ketocarotenoids have been found in high concentrations within mitochondrial membranes (Ge *et al.*, 2015; Hill *et al.*, 2019). Research on both wild and captive vertebrates and invertebrates has shown consistent connections between energy metabolism and carotenoid modification for external coloration. In birds, mitochondrial performance, metabolic rate and mitochondrial turnover is correlated with the redness of ketocarotenoid feather ornamentation (Hill *et al.*, 2019). Also in birds, direct manipulation of the redox environment through chemical changes to membrane stability result in quantifiable changes to carotenoid ketolation for red coloration (Cantarero *et al.*, 2020*a*,*b*). In both birds and crustaceans, artificially increasing the rate of redox reactions and energy expenditure results in increases to the rate at which carotenoids are modified (Fernandez-Eslava *et al.*, 2022; Powers *et al.*, 2022). These recent experiments echo observations made decades earlier from studies of the effects of captivity on feather coloration of common crossbills *Loxia curvirostra*. From these experiments, Völker (1957) hypothesized that the ability of crossbills to convert pigments from yellow to red was dependent on the rate of oxidative processes within the bird.

VIII. CONDITION DEPENDENCY IN NOVEL TRAITS

A key test of the origin of condition dependency is an assessment of whether novel ornamental traits can be immediately associated with individual condition when they first appear in a population. The resource trade-off hypothesis proposes that condition dependency can evolve under the influence of sexual selection through mechanisms to invest more resources in ornamentation. By contrast, the index hypothesis proposes that the condition dependency of traits used as ornaments does not evolve; it is an inherent property of the trait. Thus, under the index hypothesis, novel traits can be strongly linked to individual condition as soon as they exist in a population. For instance, in a population of tigers, scratching a tree as high as an individual can reach is an honest signal of body size the first time the behaviour is performed (Maynard Smith & Harper, 1995). Scratch height does not become more closely linked to condition if it becomes an assessed signal and benefits the marking tiger; it is inherently honest. By the same logic, traits with high condition dependency can exist in systems in which they are not the object of sexual selection or where they are not assessed at all. Carotenoid ornaments are the art that adorns the requisite condition-dependent spandrel of carotenoid physiology (Fig. 1).

To our knowledge, the only well-controlled study of condition dependency of a novel carotenoid ornament concerns the Yongzhang golden turtle, a domestic population of the Chinese softshell turtle (*Pelodiscus sinensis*) (Zhang *et al.*, 2022). In the wild, these softshell turtles display a dark green coloration that appears to be cryptic and not ornamental. Wild Chinese softshell turtles deposit modest levels of yellow carotenoids as part of the production of their green coloration (Wang *et al.*, 2022). Through 16 years of intense artificial selection for greater carotenoid content of tissues, agricultural researchers have transformed these drab turtles into brilliantly ornamented animals. Yongzhang golden turtles have bright yellow coloration across their entire surface integument (Wang *et al.*, 2022), which makes the turtle bright and conspicuous in a pond environment. Thus, a conspicuous colour ornamentation has evolved through intense artificial selection in only 16 generations. And, consistent with the index hypothesis, ornamental coloration is already a condition-dependent ornament in the golden turtles. Zhang *et al.* (2022) observed a strong and statistically significant positive association between carotenoid coloration and total antioxidant capacity and a strong and statistically significant negative association between yellow coloration and levels of malondialdehyde, an indicator of oxidative damage. The Yongzhang golden turtle example involves yellow coloration produced by deposition of the dietary carotenoid lutein. Production of such yellow coloration does not involve carotenoid transformation, but the uptake and transport of carotenoid to the integument could rely on redox-dependent processes. Unmodified dietary carotenoids are also linked to individual condition across numerous studies on bird coloration (reviewed in Weaver *et al.*, 2018*b*). Thus, we have condition dependency in a novel trait without selection on condition dependency.

The bright yellow coloration Yongshang golden turtles could be taken as a novel ornamental trait because it deviates from the drab green coloration of wild Chinese softshell turtles. Similar bright yellow carotenoid coloration functions as a criterion in mate choice in many other vertebrates (Hill, 2006*b*; Svensson & Wong, 2011). However, a key assumption here is that carotenoid coloration was not sexually selected in wild Chinese softshell turtles. Moreover, it is possible that resource trade-offs underlie signal honesty in the Yongshang golden turtle, although the data at hand do not suggest that this is the case. For a trade-off to underlie a condition-dependent trait, there must be a critical limiting resource (carotenoids in this case), and there is no biochemical evidence that dietary carotenoids are limiting for most wild animals (Hudon, 1994; Moller *et al*., 2000; Hadfield & Owens, 2006; Koch, Wilson & Hill, 2016*b*). As we outline herein, evidence supports explanations other than resource trade-offs underlying condition-dependent colour display in Chinese softshell turtles or other animals with carotenoid coloration. Doubtless, however, a better test of the hypothesis that carotenoid ornaments are condition dependent whenever they appear in a population would involve studies of truly novel ornamental traits.

Truly novel ornaments can be manufactured by humans by moving the gene or genes that code for an ornament from a taxon in which the trait evolved to another taxon with no history of display of the trait. Aviculturists did just that when they moved the ketolase gene (CYP2J19) that enables some birds to produce red feathers from the red siskin (*Spinus cucullata*) to the common canary – producing red factor canaries (Birkhead, 2003; Lopes *et al*., 2016). Phylogenetic reconstructions suggest that red siskins and canaries have not shared a common ancestor for about 9 million years (Arnaiz-Villena *et al*., 1999; Zuccon *et al*., 2012) and that the common ancestor of red siskins and canaries used modified yellow pigments, not red pigments, in its plumage (Ligon *et al*., 2016). Thus, through crossing and backcrossing, CYP2J19 was dropped into a genetic background in which condition dependency for red coloration could not have evolved. Nevertheless, red coloration in red factor canaries appears to be a condition-dependent trait. In controlled feeding experiments, whether red factor canaries were supplemented with β-carotene, lutein/zeaxanthin, or β-carotene/β-cryptoxanthin had a significant effect on the saturation of their feathers (Koch, Mcgraw & Hill, 2016*a*). These observations support observations of bird breeders that diet affects the expression of red coloration in red factor canaries, making it a condition-dependent trait (Birkhead, 2003). Well-controlled experiments subjecting red factor canaries to environmental challenges such as pathogen exposure are needed. Studies of novel traits created by humans through breeding or by direct transfer of genes coding for ornaments can provide important tests for hypotheses for the evolution of condition dependency.

IX. CONDITION DEPENDENCY WITHOUT SEXUAL SELECTION

In 1889, Alfred Wallace made the insightful observation that bright coloration in animal integuments cannot be assumed to exist as a result of natural or sexual selection. Wallace (1889) pointed out that internal tissues and organs of vertebrate animals are vividly coloured and that this internal coloration is certainly not subject to either natural or sexual selection. Wallace proposed that the coloration of these internal organs was ‘a necessary result of the highly complex chemical constitution of animal tissues and fluids’ (Wallace, 1889, p. 297). In more modern language, Wallace proposed that the core functions conducted by the cellular components of tissues and organs – particularly receiving and donating electrons – caused these molecules to interact with photons in such a way that they produced coloration. An implication of these ideas is that visual signals of vital cellular processes are an inevitable component of complex biological systems and that they simply await receivers to use the information.

Red carotenoid pigments play key roles in the cellular processes of animals aside from producing red coloration in animal integument. Carotenoids serving non-signalling functions provide unique opportunities to access the condition dependency of traits that cannot be subject to sexual selection. One well-known role of the red carotenoid astaxanthin is to function as a band-width filter in the cone cells in the retina in some vertebrates, thereby refining colour vision (Toomey & Corbo, 2017; Toomey & Ronald, 2021). Moreover, phylogenetic reconstructions reveal that the evolution of an enzymatic pathway to produce bright red oil droplets that play key roles the colour vision of birds and turtles long pre-dated the evolution of red coloration in the skin or feathers of birds or turtles that use the same pathway (Twyman *et al*., 2016). And these red drops of oil in the retina, which appear as brilliant ovals of intense red coloration under a microscope, are condition dependent because their redness correlates with the body condition of individuals. In a large sample of wild house finches *Haemorhous mexicanus*, Toomey & McGraw (2009) found that the concentration of astaxanthin in the retina, which is the primary determinant of redness in animal tissues, was significantly positively related to body condition. In other words, oil droplet coloration is condition dependent, and it did not evolve this condition dependency through sexual selection. Red coloration produced by this same enzymatic pathway was subsequently co-opted as a condition-dependent sexual signal (Twyman *et al*., 2016). The implication of these studies is the red carotenoid coloration that is the product of modification of yellow dietary pigments will be condition dependent wherever it occurs, because the production of such pigments is inherently condition dependent.

Another circumstance that allows for tests of the resource trade-off *versus* the index hypothesis for the origin of condition dependency is when ornamental traits exist in animals that cannot perceive the trait. For instance, if the amplitude or rhythm of the rattle of a rattlesnake was closely associated with neuro-muscular function of an individual, but that species of snake could not hear the rattle, then the rattle sound could not have been shaped by sexual selection. Such a test for condition-dependent signalling, where there is no sexual selection for the trait, was conducted in a series of studies of marine copepods in the genus *Tigriopus*. These copepods deposit keto-carotenoid pigments in their body tissue giving them brilliant red coloration that, to a human observer, appears to be an ornamental trait like red feathers in a bird or red skin in a fish (Weaver *et al*., 2018*a*). The physiological mechanisms that give rise to red integumentary pigmentation in copepods are very similar to the mechanisms for red integumentary pigmentation in songbirds (Passeriformes). In both *Tigriopus* copepods and songbirds, individuals ingest yellow carotenoid pigments in their diets and use enzymes to convert the yellow dietary pigments to red pigments used to colour their integuments (Weaver *et al*., 2018*a*).

A key aspect of red coloration in *Tigriopus* copepods is that, although the coloration appears ornamental, it is not subject to sexual selection. First, it is highly unlikely that copepods have the capacity to perceive differences in the intensity of red coloration in potential mates. *Tigriopus* copepods have a simple eyespot, not an eye that can form an image, and their neural capacity is very likely too restrictive to allow them to assess subtle colour differences between potential mates, even if such visual information was available (Martin, Speekmann & Beidler, 2000; Andrew, Brown & Strausfeld, 2012). Evidence suggests that copepods cannot perceive red light, much less discriminate between potential mates that are more or less red. Nevertheless, Powers, Hill & Weaver (2020) conducted carefully controlled behavioural studies to test for possible assessment of coloration during mate choice. Males are likely the choosing sex in *Tigriopus* copepods because males pursue and clasp females (Burton, 1985). Powers *et al*. (2020) conducted experiments in which male *Tigriopus californicus* copepods were given a choice between females that were bright red *versus* females that had no red coloration. Males showed no significant preference for red *versus* colourless females. Thus, both the anatomy of the eyespots of copepods and mate choice trials indicate that there is no sexual selection for red coloration in *Tigriopus* copepods. Experimental evidence indicates that keto-carotenoids in the tissues of *Tigriopus* copepods helps protect tissues from damage from pro-oxidant exposure (Weaver *et al*., 2018*c*), but redness, *per se*, serves no function in *Tigriopus* copepods.

Even though red coloration is not subject to sexual selection, it is nevertheless a condition-dependent signal of individual health and function. The condition dependency of red coloration in copepods was tested in a study of *Tigriopus japonicus* by exposing one group of individuals to copper (Cu^{2+} from $CuSO_4$) while maintaining a second group under the same conditions but without exposure to copper (Weaver *et al*., 2016). Copper is an important pollutant in marine environments and is well known to affect the fitness of animals negatively (Eisler, 1998; Main, Ross & Bielmyer, 2010). Copper exposure caused a significant up-regulation of the antioxidant gene glutathione reductase in copepods, demonstrating that copper exposure caused oxidative stress – one well-accepted form of low condition. The copper-exposed group also showed reduced production of the red carotenoid astaxanthin and reduced red coloration compared to the control group (Weaver *et al*., 2016). Caramujo *et al*. (2012) also showed a similar response to copper and ultraviolet (UV) exposure in the meiobenthic copepod *Amphiascoides atopus*. Hence in copepods, red coloration is condition dependent and reflects the health state of individuals without sexual selection.

In another study assessing the condition dependency of red coloration in copepods, Powers *et al*. (2021) tested hybrid inbred lines of *Tigriopus californicus* from crosses between and within populations. Line-specific variation in respiratory function in *T. californicus* produced by crossing genetically divergent populations has been studied in detail and linked to specific functional incompatibilities in the products of nuclear and mitochondrial genes that code for components of the electron transport system or the transcriptional or translational mechanisms that support production of the electron transport system (Burton, Pereira & Barreto, 2013; Barreto *et al*., 2018). Hybrid dysfunction creates low-condition individuals. Powers *et al*. (2021) looked at production of astaxanthin across previously established, multigenerational hybrid and non-hybrid lines of copepods and found that individuals within hybrid inbred lines produced less astaxanthin when provided with dietary carotenoids when compared to non-hybrid inbred lines. In a another cross between southern and northern Californian populations, they also observed a correlative decrease in energy production, reproductive performance, and carotenoid modification by the third hybrid generation compared to original parental population individuals (Powers *et al*., 2021). These results indicated that changes in cellular metabolism are associated with changes in carotenoid modification, even in species in which coloration serves no behavioural purpose.

X. ORNAMENTATION IN A PETRI DISH

The discovery of key genes involved in the production of red pigments in vertebrates provides novel opportunities to study the production of red pigments in cell culture (Hill, 2022). Indeed the genes involved in production of the modified carotenoid pigments used in ornamentation can be transfected into cultured cells from organisms lacking carotenoid ornamentation or any possible ancestral history of carotenoid ornamentation (Toomey *et al*., 2022). Such a system provides a clean test of hypotheses for the evolution of condition dependency because red coloration is produced in a cellular environment that did not evolve but that was constructed by biologists. To increase the impact of such studies, cell lines may be cultivated from several tissues used to produce carotenoid ornamentation. No studies of condition-dependent colour production by cultured cells have yet been published, but observations of whether or not ornament-producing genes are dependent on the condition of a cell culture would provide clean tests of the hypotheses for the origin and maintenance of honest signals of condition.

XI. DISCUSSION

Despite the importance of the concept of condition-dependent signalling to sexual selection theory (Maynard Smith, 1991; Andersson & Simmons, 2006), there is no consensus on the origin of condition-dependent expression of ornamental traits, nor on how signal honesty is maintained over evolutionary time (e.g. Bortolotti *et al.*, 2009; Higham, 2014; Tibbetts, 2014; Weaver *et al.*, 2017). The hypothesis that condition dependency evolves under the influence of sexual selection (Rowe & Houle, 1996) has proved challenging to test. Difficulties arise, first, because evolutionary biologists have been unable to observe the process of sexual selection and the evolution of ornaments either in laboratory or wild animals (Bonduriansky & Rowe, 2005; Bussiere *et al.*, 2008). Second, with few exceptions, evolutionary biologists lack a full understanding of the mechanisms of production of ornamental traits and the gene products involved in the processes. The result is that, like tourists marvelling at the ornate spandrels of a cathedral, researchers have been left with observations of evolved systems and speculation about the mechanisms that might underlie observed ornamental traits.

Rapid advances in tools for linking phenotypes to genotypes (Funk & Taylor, 2019; Price-Waldman & Stoddard, 2021), the capacity for gene editing (Sun *et al.*, 2020), and synthetic biology (Davies, 2017) are providing fantastic new opportunities for reassessing old questions regarding ornament evolution. Each of these tools is advancing understanding of sexual signalling involving red and yellow carotenoid coloration, and emerging observations from disparate approaches to the study of carotenoid coloration do not support the resource trade-off hypothesis. There is little evidence supporting the hypothesis that trade-offs in resource allocation underlie signal honesty and a growing body of data, including that study of systems with knockouts of key genes in the carotenoid-production systems, indicate that this hypothesis does not provide an accurate description of how carotenoids are distributed within the bodies of vertebrates (Costantini & Møller, 2008; Koch *et al.*, 2018, 2019). If resource limitation does not underlie signal honesty, then a key assumption of the resource trade-off hypothesis is falsified as is the hypothesis that condition dependency evolves *via* sexual selection.

In addition, there is growing evidence that carotenoid ornaments are inherently honest signals (Hill, 2011; Weaver *et al.*, 2017; Powers & Hill, 2021). Colour displays created with carotenoids are instantly condition dependent when they arise as novel traits in a population (Zhang *et al.*, 2022). In animals that lack the sensory capacity to assess colour ornaments, red carotenoid coloration is nevertheless condition dependent (Weaver *et al.*, 2016; Powers *et al.*, 2021). Observations from cell culture studies are particularly promising because they hold the potential to test key questions related to honest signalling rigorously: how many genes are involved in the production of a carotenoid ornament? Is sexual selection necessary for evolution of the mechanisms of production of carotenoid-based colour displays? Is red coloration produced by ketolated carotenoids an inherently condition-dependent trait? We are on the cusp of such studies of sexual selection in cell culture (Toomey *et al.*, 2022). In this review, we focused on carotenoid ornamentation. The same questions about the origin of condition dependency can be asked of any ornamental trait whose expression is linked to individual quality. We propose that the resource trade-off hypothesis is founded on unsupported assumptions and should be invoked cautiously as an explanation for the origins of condition dependency for any ornamental trait. These are testable ideas and future research will settle disputes over the value of different theories.

Almost 50 years ago, the great evolutionary biologists Stephen J. Gould and Richard Lewontin cautioned fellow evolutionary biologists regarding the pitfalls of adaptive story telling. Gould and Lewontin focused on the origin of traits in organisms. In our opinion, the message of Gould and Lewontin applies equally to explanations of the condition dependency of ornaments. It is unwise to assume adaptive evolution, and the data at hand suggest that carotenoid ornaments do not evolve to function as honest signals – they are inherently reliable signals of cellular function.

XII. CONCLUSIONS

(1) In many animals, red and yellow carotenoid coloration has been shown to be a condition-dependent signal of quality, but the origin of the condition dependency of ornamental traits is a focus of debate. The resource trade-off hypothesis proposes that condition dependency evolved in response to sexual selection on the use of limited carotenoid resources while the index hypothesis proposes that production of carotenoid coloration is tied to vital cellular processes and is inherently condition dependent.

(2) A key prediction of the resource trade-off hypothesis but not the index hypothesis is that the link between individual condition and carotenoid coloration arises from trade-offs in use of carotenoids for ornamentation *versus* body maintenance. Evidence for such trade-offs is weak and experiments with genetically modified birds refutes the trade-off hypothesis.

(3) Consistent with predictions of the index hypothesis but not the resource trade-off hypothesis, carotenoid coloration is condition dependent even when it is not subject to natural or sexual selection, and visible carotenoid coloration is condition dependent as soon as it appears in a population.

(4) Manipulation of genes that function in the production of carotenoid coloration and the expression of ornamental traits in synthetic biological systems is providing exciting new opportunities for studying condition-dependent signalling.

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