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Evolution: The biochemistry of honest sexual signaling

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The discovery of a new enzyme required for production of red carotenoid pigments in vertebrates provides insights for how shared biochemical pathways may be the key to understanding honest signaling via plumage coloration.

The flashy colors and outrageous behavioral displays of animals are the delight of naturalists but the bane of evolutionary biologists. Such extravagances, which come at a cost of survival, seem inconsistent with natural selection. A primary explanation for male ornaments is that female choice for signals of male quality — so-called 'honest signals' — drives their evolution[1]. Indeed, in many birds (Figure 1), reptiles and fish, the yellow or red coloration produced by carotenoid pigmentation signals health and vitality[2,3]. Despite decades of study, however, there is little consensus regarding the mechanisms that could link the brilliance of red or yellow color displays to fundamental traits, such as disease resistance, endurance or cognition[4]. Unlike melanin or pteridine pigmentation, nanostructures and other sources of integumentary coloration, carotenoids cannot be produced endogenously by vertebrates; they must be ingested. Moreover, most vertebrates ingest only yellow carotenoid pigments, but to produce red coloration, dietary yellow pigments, such as lutein and zeaxanthin, must be converted to red ketocarotenoids[5]. Hence, the story of honest signaling via red pigmentation is largely a tale of enzyme function. In this issue of *Current Biology*, Matthew Toomey, Miguel Carneiro, Joseph Corbo and colleagues[6] add a key biochemical piece to the puzzle of how animals transform yellow dietary pigments to red pigments used in displays. This discovery has important implications for how simple carotenoid-based color displays of skin and feathers can accurately summarize the quality of an individual while resisting the invasion of cheating strategies.

Until recently, researchers studying animal coloration had essentially no information regarding the enzymes that give rise to carotenoid coloration. A series of studies in the past decade culminated in the discovery of CYP2J19, an enzyme purported to produce red ketocarotenoids from yellow carotenoid precursors and the presumed source for most red skin and feather coloration in birds and turtles[7,8]. Knowledge of CYP2J19, a member of a class of P450 enzymes that can be targeted to mitochondria, led to a focus on mitochondrial function in relation to pigment production[9,10] and supported a shared pathway hypothesis wherein pigment production is intimately linked to vital cellular processes — in this case aerobic respiration in the mitochondria[4]. Linking pigment production to cellular respiration potentially explains how a trait as seemingly simple and frivolous as feather coloration could reliably signal aspects of animal performance as diverse as health, endurance and cognition[11].

An emerging theory posits that the strong associations between carotenoid coloration and individual quality arise from co-dependency of CYP2J19 and mitochondrial function, but questions and doubts remain. Integumentary ornaments

Figure 1. The cardinal link between color and condition.
A male Northern Cardinal (*Cardinalis cardinalis*) feeding his male offspring. The young cardinal is growing feathers with red pigments, a process that requires two enzymes to change the yellow pigments in food to the red pigments used in feathers (photo: Geoffrey Hill).

produced by yellow canary xanthophylls, which are a product of metabolic conversion of yellow dietary pigments not involving CYP2J19, showed the same condition-dependence as red ornaments[3]. A recent study found no association between CYP2J19 gene expression and male feather coloration in a red songbird[12]. Moreover, canary xanthophylls are often co-expressed with red ketocarotenoids in the integument of red animals, with no obvious function[5]. Phylogenetic reconstructions of clades of birds indicated that there have been many transitions between plumage pigmented with yellow canary xanthophylls versus red ketocarotenoids[13,14]. The fluidity of these transitions suggests that both forms of coloration might convey similar information.

With their new study, Toomey and colleagues[6] demonstrate that the production of red ketocarotenoids from yellow dietary pigments requires two key enzymes, not just CYP2J19 as had been presumed. The missing enzyme is 3-hydroxybutyrate dehydrogenase 1-like (BDH1L), which catalyzes the oxidation of the dietary pigments lutein and zeaxanthin to canary xanthophylls — a yellow-to-yellow pigment conversion. The key observation, which Toomey and colleagues[6] made in cell-culture experiments, is that CYP2J19 requires the products of BDH1L to produce red pigments. Thus, the actions of both CYP2J19 and BDH1L are required to produce red ketocarotenoids. The ketolation of dietary carotenoids is a two-step process, not a one-step process as had been presumed.

Along with the discovery of BDH1L as a key missing enzyme for the production of red pigments in birds and fish, Toomey and colleagues[6] traced the genetic source of an orange variant of an otherwise red species, the red-throated parrotfinch (*Erythrura psittacea*). They found that a mutant version of the gene *TTC39B* was perfectly associated with the orange phenotype. Furthermore, they found that TTC39B substantially enhanced the production of red ketocarotenoids in cell culture when co-transfected with CYP2J19 and BDH1L. TTC39B has the potential to explain variation in the degree of integumentary pigmentation, compared to the red/yellow on/off switch of BDH1L/CYP2J19.

What do the revelations of Toomey and colleagues[6] mean for understanding how red coloration can serve as an honest signal? It was previously proposed that CYP2J19 may be localized in the inner mitochondrial membrane and thereby connect red coloration to mitochondrial function[9], but the authors note that P450 enzymes like CYP2J19 require the sequential donation of two electrons to perform their catalytic function. Either NADH or NADPH are likely to act as electron donors. Toomey and colleagues[6] found that enzymes involved in the NADH, but not the NADPH, pathway are highly enriched in cells where CYP2J19 is active, compared to other cell types, where CYP2J19 is absent. If CYP2J19 does indeed receive electons from NADH, it is likely localized in the endoplasmic reticulum, not the mitochondria[15]. Interestingly, BDH1, which is structurally very similar to BDH1L, is targeted to mitochondria, and BDH1 oxidoreductase activity modulates mitochondrial redox potential[16]. Thus, BDH1L may be targeted to the mitochondrion, thereby creating a functional link between BDH1L activity (and hence feather coloration) and mitochondrial respiration. The site of action of TTC39B is unknown, and it will be interesting to see how regulation of this color promotor is tied to mechanisms of yellow and red pigment metabolism.

With the discovery of BDH1L as a key pigmentary enzyme in the production of red pigments in vertebrates, the doors are flung open for new hypotheses regarding the association between carotenoid pigmentation and individual condition. One speculation is that if the conversion of dietary pigments to canary xanthophylls via BDH1L occurs in the mitochondria, or is otherwise tightly linked to aerobic respiration, then enzymatic conversions involving BDH1L and not CYP2J19 may be the condition-dependent step in production of carotenoid coloration. This would explain why, in a recent study of red feather coloration in weaver birds, expression of CYP2J19 was not tightly associated with individual condition[12]. Alternatively, the activity of both CYP2J19 and BDH1L might be linked to mitochondrial function, even if one or both localize in the endoplasmic reticulum, because the regulation of aerobic cellular respiration is closely tied to the endoplasmic reticulum[17]. Either hypothesis would explain why pigmentation with either red keto-carotenoids or yellow canary xanthophylls is linked to condition — both require conversion by BDH1L. With growing knowledge of the enzymatic pathways that underlie animal coloration,

key experiments to elucidate the biochemical basis of honest signaling are now possible. It is an exciting time to be working in the field of animal coloration.

DECLARATION OF INTERESTS

The author declares no competing interests.

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Worm development: Push not pull in gonad morphogenesis

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How does tissue elongation occur? A recent paper identifies a new mechanism: elongation of the *Caenorhabditis elegans* hermaphrodite gonad is driven by pressure from proliferating germ cells confined within a tube. The distal tip cell, which caps the tube, remodels the extracellular matrix and adjusts cell–matrix adhesion to guide the way.

Historians have long pondered the question: what is the relationship between the leader and the led? *Caenorhabditis elegans* biologists, on the other hand, have always been fairly confident about the leadership role of the distal tip cell (DTC) in guiding gonad morphogenesis, assuming the germ cells to be passive followers. A recent paper in *Developmental Cell* from Ronen Zaidel-Bar and colleagues[1] challenges this view, proposing instead a more complex relationship between leader and followers, one in which the follower germ cells provide propulsion and the leader DTC is responsible for navigation.

In the *C. elegans* hermaphrodite, the gonad develops from a four-cell primordium into a mirror-image, bilobed, U-shaped gonad over the course of the four phases of larval development. The gonad elongates along the ventral body wall muscle, then makes a 180° turn, extending toward the animal's midline by the end of larval stage 4 (L4; Figure 1A). The proliferating germ cells are surrounded by a basement membrane, enwrapped by somatic cells called sheath cells, and capped by the DTCs, which cover the end of each