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Chapter 11

Signal Functions of Carotenoid Colouration

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A. Introduction

The importance of carotenoids for natural colouration, in relation to other classes of pigments and structural colours, has been outlined in *Chapter 10*. But colour only has significance if it is perceived, identified and interpreted by other organisms (animals). In other words, colour is a means of communication, a signal. Now, in this Chapter, this new direction for carotenoid research, behavioural ecology, is highlighted. Various hypotheses that have been proposed to explain the signal functions of colour, and particularly of carotenoids, in plants and animals are discussed and the empirical evidence to support these hypotheses is presented.

1. Biological signals and their reliability

A biological signal can be defined as any morphological, behavioural, or chemical feature that has evolved because it alters the behaviour of other individuals. A signal may therefore convey information, about the current state of the signaller or its future behaviour, which is of relevance to the receiver [1]. The information contained in signals need not always be correct, but it must be correct often enough for selection to predispose the receiver to respond to it [1]. Otherwise, at some point the signal will be ignored because it has lost its meaning. This raises the question of what maintains the reliability, or ‘honesty’ of signals? The ‘handicap principle’ suggests that signals are honest because they are costly to produce [2]. For example, the tail of a peacock (*Pavo cristatus*) might be costly to grow in terms of resource utilization, or it might be costly to bear because it impedes efficient flight and therefore makes the bird more vulnerable to predation. So individuals with longer tails should be higher quality individuals, because relatively poor quality individuals should not be able to afford to pay

1 such high costs of resource allocation or predation risk [2]. The handicap principle has been
2 evoked to explain the evolution of a broad range of signal types, some of which are based on
3 colouration.

4 In certain instances, however, signals need not be costly to be reliable. If the interests of
5 the signaller and receiver coincide, then signals can be honest. For example, the bright red,
6 warningly coloured toxic insect and its potential predator both benefit if the insect is not
7 attacked. Secondly, certain signals are honest because it is impossible to lie, namely indices of
8 quality such as body size [1].

10 2. Carotenoids as signals

11
12 For a resource to be subject to allocation trade-offs, as envisaged in the handicap principle, it
13 must (i) be needed for more than one body function, and (ii) be in limited supply. Carotenoids
14 fulfil these criteria, because they have a variety of other functions as well as being pigments
15 responsible for colouration. In addition, the physiological supply of carotenoids may be
16 limiting because, at least in animals, these compounds cannot be produced *de novo* and must
17 be obtained in the diet [3]. Indeed carotenoid colouration abounds in Nature in many different
18 signalling contexts. In animals, carotenoid-based signals include those designed for
19 communication both between species (*e.g.* warning colouration) and within species (*e.g.*
20 epigamic colouration – colours associated with courtship). In contrast, the signal functions of
21 carotenoids in plants have been relatively little studied.

23 **B. Carotenoid Signals in Plants**

25 1. Fruits and flowers

26
27 Reproductive structures of plants, *i.e.* fruits and flowers, use colour and odour to attract
28 consumers who then serve to disperse the seeds or pollen. Compared to animal signals, plant
29 colour signals are generally simpler in expression, because they lack a behavioural component
30 and are displayed against a relatively unchanging background [4]. This simplicity makes plants
31 good models for studying signal evolution. Little is known, however, about evolutionary
32 changes to optimize the attractiveness of flower colour to animals, or evolutionary changes in
33 animals' visual systems or behavioural responses to plant colouration [5]. Moreover, little is
34 known of the signal functions of carotenoids, as a specific class of pigments, in fruits and
35 flowers. Most yellow and orange, and some red reproductive structures in plants are coloured
36 by carotenoids, whereas pink, purple, blue-black and often red fruits and flowers use
37 anthocyanins [6]. However, no study has related the carotenoid content of any particular fruit

1 or flower colour signal to any aspect of animal behaviour. Carotenoids in many pollens may
2 serve to improve pollen detectability [6], but there is no empirical evidence to support this. In
3 fruits and flowers, carotenoids may perform several functions independent of signalling, such
4 as photoprotection (*Chapter 10*), and they play essential roles in photosynthesis (*Chapter*
5 *14*).

6 Choices made by consumers between different fruits or flowers are based on several
7 factors, including innate preference, past experience of relative rewards, familiarity or ease of
8 handling, and sensory adaptations and constraints that may make them more or less able to
9 detect particular colours or odours. One hypothesis for the diversity of flower and fruit
10 colouration proposes that particular classes of consumers are associated with particular colour
11 signals, because pollination or seed dispersal is most efficient when done by specific animal
12 taxa [5]. For example, pipevine swallowtail butterflies (*Battus philenor*) innately prefer yellow
13 colour, and to a lesser extent blue and purple [7], whereas Indian red admirals (*Vanessa indica*)
14 prefer yellow and blue [8]. However, learning can play an important role in determining colour
15 preferences. It has classically been considered that red flowers are innately attractive to
16 hummingbirds and simultaneously invisible to bees but, in fact, hummingbirds' colour
17 preferences are learned and based on nectar rewards [9], and this typically leads to preferences
18 for red flowers (at least in N. America) because these provide the greatest nectar rewards in
19 those regions where hummingbirds live [10]. Bees do visit red flowers [11], but take longer to
20 locate red than blue flowers because red contrasts relatively poorly against the background as
21 perceived by the bees. Red floral colour may therefore be a strategy to reduce detectability by
22 bees [5]. Because peak visual sensitivities are species-specific, there is some scope for
23 directing signals at particular taxa, or avoiding others.

24 Fruit and flower colour is expressed maximally when a fruit is ripe or a flower fully open,
25 *i.e.* when seed viability or nectar availability is highest [4]. But do such signals provide an
26 honest indicator of the nutritional reward to consumers, *e.g.* availability of nectar, or energy?
27 Few studies have addressed this question. In the sky lupin (*Lupinus nanus*), the petal banner
28 spot changes from white to red once pollination has taken place, and red colour increases the
29 attractiveness of the floral display, so optimizing the foraging and pollination efficiency of
30 consumers [12]. Nutritional components, including lipid, protein, carbohydrate and water
31 content, were found not to correlate with colouration indices in red or black fruits, however,
32 but did so in fruits of other colours such as blue and green [13]. Conspicuous colours including
33 red and black may, therefore, signal fruit detectability whereas less conspicuous colours (*e.g.*
34 blue or green) may signal fruit nutritive reward. Similarly, fruit nutritional components,
35 including mass, energy and sugar concentration, did not correlate with colour scored
36 qualitatively by human observers [14]. The possibility that the pigments responsible for fruit
37 colour may themselves be a valuable nutritional reward to consumers was not considered,

1 however. Indeed, the brightly coloured seeds of *Margaritaria* spp. have been suggested to be a
2 dishonest signal because they offer no nutritional reward to the consumer [4], but this
3 overlooks the possible nutritional value of carotenoids or other pigments [15]. Do foragers
4 seek fruits and flowers richer in carotenoids? Carotenoids are a potentially limiting resource
5 for animals (see Section D). What is the potential cost to fruits and plants of allocating
6 carotenoids to signal production as opposed to other somatic or reproductive functions, and is
7 this balanced by benefits of more efficient seed or pollen dispersal?

8 An alternative hypothesis, that such colour displays may serve to render invertebrate
9 herbivores (*i.e.* parasites) more conspicuous to predators and therefore lead them to avoid
10 foraging on such plants [16], has not been tested experimentally.

11 12 2. Leaves

13
14 Why do leaves in many deciduous tree species become yellow or red in autumn? The
15 conventional explanation is that such colour (which is due to the presence of carotenoids and
16 anthocyanins) is a non-adaptive consequence of leaf senescence; as chlorophyll is degraded,
17 the underlying carotenoids are exposed and large amounts of red anthocyanins may be
18 synthesized. This spectacular autumn leaf colour may perform a signalling role, having arisen
19 because of co-evolution between trees and insects [17,18]. Red and yellow leaf colour may
20 make the defensive commitment of the plant clear to insects (*e.g.* aphids) that infest the plant
21 in autumn and exploit it as a host for the winter [17]. Well-defended plants may thus reduce
22 their parasite load, and the parasites benefit by infesting a more exploitable host. Because such
23 allocation of carotenoids and anthocyanins to leaves may be costly to plants, autumn leaf
24 colouration is suggested to be an honest (handicap) signal of plant defensive capacity [17].

25 Those plant species that suffer greater exploitation by insects should therefore invest more
26 resources in defence and signalling of such defence [17,18]. A comparative study has indeed
27 shown a positive association between the degree of autumn colouration and the diversity of
28 monophagous aphids across 262 species of deciduous trees [17]. The most effectively
29 defended individuals of a species should produce the most intense colour and be the most
30 likely to be avoided by parasitic insects [17,18]. Indeed, it has been shown that individuals of
31 mountain birch (*Betula pubescens*) in better condition produce more intense autumn
32 colouration [19,20] and suffer less insect damage the following season [19], and *Prunus padus*
33 trees with a higher percentage of red-yellow leaves were colonized by fewer aphids
34 (*Rhopalosiphum padi*) [21]. All these studies are correlational, however, and an experimental
35 approach is required to demonstrate cause-effect relationships. It would be valuable to test
36 directly whether aphids are averse to the colour yellow [21], or attracted to it [18]. And, of
37 course, alternative explanations for autumn leaf colour (*e.g.* photoprotection) may not be

1 mutually exclusive [22].

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4 **C. Carotenoid Signals in Animals**

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6 1. Signalling to other species (heterospecifics)

7

8 a) Species recognition

9

10 Amongst bird species, for example, there is tremendous variation in the colour of plumage and
11 bare parts (*e.g.* skin). The traditional explanation is that such colour variation has evolved to
12 enable different species to recognize (and avoid) each other to reduce the risk of hybridization
13 [23,24]. The morphological or ecological differences between closely related species should,
14 therefore, increase (diverge) in sympatric species, *i.e.* those that occur in the same area, a
15 phenomenon called ‘character displacement’. This prediction has been supported by
16 comparisons amongst sympatric *Ficedula* species of flycatchers in Europe [25], which
17 display melanin colouration, and amongst *Agelaius* species of blackbirds in North America
18 [26]. Tricoloured blackbirds (*A. tricolour*) and red-winged blackbirds (*A. phoeniceus*) have
19 striking red and yellow, or red and white, epaulette feather colour, respectively [26]. In *A.*
20 *phoeniceus*, red epaulettes have been shown to contain astaxanthin (**404-6**), canthaxanthin
21 (**380**), lutein (**133**) and zeaxanthin (**119**) [27] (carotenoids are presumably also responsible for
22 the red colour in *A. tricolour*, though this has not been verified). Apart from a few classic
23 examples, however, such character displacement does not appear to be a widespread
24 phenomenon. A recent comparative analysis concluded that, in general, sympatric species are
25 not more divergent in respect of plumage colour than would be expected by chance [28].
26 Instead, between-species differences in plumage colour were associated with the habitats
27 (particularly the lighting conditions) in which the birds live [28]. Differences in signalling
28 conditions may have led plumage colour to be adapted to provide maximum chromatic
29 contrast against those backgrounds and in those light environments that are relevant to
30 individual species [2,29]. Between-species differences in colour may also have evolved to
31 enable individuals of the same species to recognize each other, rather than to avoid members of
32 other species [30]. Importantly, signal traits under selection for species recognition may have
33 evolved for a different purpose; for example, the strikingly coloured epaulettes in *Agelaius*
34 blackbirds function in social status signalling.

35 Due to practical and technical constraints, data used in large-scale comparative analyses
36 relate to feather colour (being obtained from museum specimens) [28] to the neglect of bare
37 parts such as legs, wattles and gapes, which could in theory have evolved to facilitate species

1 isolation. This could be an important caveat because carotenoid colouration in birds is more
2 common in bare parts than in plumage [31]. There is also a dearth of information about how
3 species differences in colouration may relate to sympatry *etc.* in taxa other than birds.

4 5 b) Warning (aposematic) colouration and mimicry

6
7 An important evolutionary mechanism thought to be responsible for conspicuous colour and
8 pattern in animals is aposematism, where appearance advertises unprofitability
9 (unpalatability, toxicity, or ability to evade capture) to predators. Carotenoids are responsible
10 for some such signals in a wide diversity of organisms including insects, amphibians, reptiles
11 and birds [32-34] but, typically, relatively few species have been studied in detail. Seven-spot
12 ladybird beetles (*Coccinella septempunctata*) have bright red elytra (wing cases) coloured by
13 several carotenoids including torulene (**11**) and β -carotene (**3**) [35]. When threatened, they
14 discharge from leg joints fluid (reflex blood) which contains an alkaloid (coccineline) that is
15 distasteful and toxic to some bird predators [36].

16 Several aposematic butterfly and moth species have been analysed for carotenoid profiles,
17 for example small ermine moth (*Yponomeuta mahalebella*), magpie moth (*Abraxas*
18 *grossulariata*) and cinnabar moth (*Tyria jacobaeae*) [37], swallowtail butterflies (*Pachliopta*
19 *aristolochiae*, *Battus philenor* and *B. polydamas*) [38], and narrow-bordered five-spot burnet
20 moth (*Zygaena lonicerae*) [39]. It is impossible, however, to know whether carotenoids play
21 any role in colour signalling, because the carotenoids were extracted from whole carcasses.
22 Carotenoids contribute to aposematic colouration in some Lepidopteran species [40]. For
23 example, kite swallowtail butterflies (*Graphium* spp.) circulate lutein (**133**) in their wing
24 veins, apparently giving yellow-green or emerald colouration [41]. The accumulation is
25 selective; with *Graphium* species, for example, lutein is the only one of the eight carotenoids
26 found in the leaves of its food plant (*Cinnamomum camphora*) that appears in the wings [41].
27 Caterpillars of the large white butterfly (*Pieris brassicae*) reared on a carotenoid-free diet lose
28 their ability to develop background-contrasting aposematic colouration. Addition of lutein to
29 the caterpillars' diet [42] restores the aposematic colouration even though this is due to
30 melanin and bile pigments as well as carotenoids in the cuticle and epidermis, suggesting that
31 dietary lutein plays an important role in mediating the endocrine response which gives rise to
32 melanization and bile pigment accumulation [42].

33 Among vertebrates, poison frogs (Dendrobatidae) produce some of the most toxic alkaloid
34 poisons known, and many species have bright aposematic colouration [43,44], at least some
35 of which is due to carotenoids [40]. In birds, chemical defence, presumably against predators
36 and/or parasites, has been described only in five species of the genus *Pitohui* and one species
37 of a separate genus (*Ifrita kowaldi*), all of which occur in New Guinea. These species possess

1 in their feathers batrachotoxin, the same potent neurotoxic alkaloid used by some poison frogs
2 of the genus *Phyllobates* [45]. Pitohuis have bright orange feather colouration, but it is not
3 known whether this is carotenoid-based or phaeomelanin.

4 Mimicry (copying the colour or pattern of a more dangerous species) is a form of colour
5 signalling related to aposematism, and may also confer defence against predation, but there are
6 no confirmed reports of the use of carotenoids for this purpose.

7 8 c) Crypsis

9
10 Animals that are camouflaged in their environment, so that detection of them is challenging, are
11 described as being cryptic. It may seem unlikely that brightly coloured carotenoids should
12 confer crypsis to animals. However, experiments have shown that long-jawed goby
13 (*Gillichthys mirabilis*), greenfish (*Girella nigricans*), and Pacific killifish (*Fundulus*
14 *parvipinnis*), when kept in tanks against different coloured backgrounds (e.g. white, yellow,
15 black) for considerable periods of time (up to 2 months), gradually changed their skin colour
16 towards that of the background [40]. Analysis showed no significant differences in carotenoid
17 content between individuals that had been exposed to control (white) and coloured
18 backgrounds; the colour changes were due only to expansion or contraction of the pigment
19 masses within the xanthophores [40] (see *Chapter 10*). These, however, were studies of
20 captive animals against artificially coloured backgrounds, and the conclusion that colour
21 changes are adaptive and confer crypsis is speculation. It is not known whether any prey
22 species in the wild reduce their carotenoid colour signals when at risk from predators.
23 Copepods may reduce their carotenoid reserves when exposed to predators [46], but a signal
24 function has not been determined for carotenoid colouration in these taxa. It has been
25 speculated that tunaxanthin (**149**) in the skin of Trinidadian guppies (*Poecilia reticulata*) may
26 play a role in crypsis [47].

27 In some insects, cryptic colouration is produced by a combination of carotenoids with
28 other pigments. For example, the stick insect *Dixippus morosus* uses α -carotene (**7**) and β -
29 carotene (**3**) together with an undetermined 'blue compound' to produce green colouration
30 [40]. There are examples of species where carotenoids facilitate crypsis, rather than being
31 directly responsible for it. In certain deep sea animals (fish, cephalopods, and euphausiid and
32 decapod shrimps) that use bioluminescent signals, the bioluminescent cells form part of a
33 complex organ (photophore) which incorporates different pigments that either reflect or
34 absorb light, and is designed to rotate to modify the direction, intensity, spectral or angular
35 distribution of the emitted light [48]. Carotenoids are located in the sheath cells of the
36 photophores and are presumed to prevent lateral leakage of light; astaxanthin (**406**) and its
37 esters are responsible for this function in the decapod shrimps *Oplophorus* and *Systemaspis*.

1 The photophores are most abundant on the animals' ventral surface, where luminescence is
2 directed downwards to match closely the characteristics of the light penetrating from the sea
3 surface. This obscures the silhouette that would otherwise be visible from below [48,49].

4 Carotenoid-protein complexes (*Chapter 6*) can produce colours such as blue, green and
5 grey in marine animals, including certain amphipods and crustaceans. For example, American
6 lobsters (*Homarus americanus*) are predominantly almost black due to a carotenoid-protein
7 complex [40]. Such carotenoid-protein complexes could confer crypsis, though this has not
8 been investigated.

10 2. Signalling to members of the same species (conspecifics)

12 a) Sexual signalling

14 Most animals carefully select the individuals with which they mate and, in many instances,
15 pursue mates of the highest quality, in order to maximize the number and quality of offspring
16 they produce [50]. Colours (often carotenoid-based) are among the many traits, including
17 songs, dances, pheromones, horns/antlers/spurs, and body size/symmetry, on which animals
18 base their mating decisions [51]. Females are typically the more selective sex when it comes to
19 mating, due to their larger investment in gametes (eggs), and thus males have developed traits
20 like bright colours to attract female mates [52]. This sexual dichromatism (colour differences
21 between the sexes) is often (but not always) a first indication that sexual selection by female
22 mate choice is driving extravagant displays in male animals.

23 Although the first evidence for a sexual-signalling role of carotenoid-based colours in male
24 animals came from studies in the early 1900s (*e.g.* in three-spined sticklebacks *Gasterosteus*
25 *aculeatus* [53]), it has been only within the past few decades that evolutionary theories of
26 mate choice have been widely accepted, mathematically modelled, tested, and ultimately
27 validated. Pioneering work [54] on carotenoid pigmentation in the orange skin of Trinidadian
28 guppies (*Poecilia reticulata*) stimulated research on sexual selection for carotenoid
29 pigmentation in fishes and birds [55]. Direct mate selection, independent of other processes
30 such as intrasexual competition, is a difficult behaviour to observe in the field, but some
31 examples, namely two fish species, one lizard species, and seven bird species, where careful
32 experimental studies have shown that females choose mates based on variation in the extent or
33 quality of male colours that are confirmed to be carotenoid-derived, are listed in Table 1.
34 These studies are of different kinds (*e.g.* field *v.* lab, correlational *v.* experimental), but their
35 results are consistent with the notion that brighter or more extensive colours are sexually
36 favoured. There are other studies on mate choice for bright colouration in these taxa [reviewed
37 in 56,57] but, in these cases, the chemical basis for the colour has not been identified. Evidence

1 is lacking for a sexual signalling role of carotenoid colours in invertebrates, amphibians, and
2 mammals, which are less commonly sexually dichromatic. In contrast, in some birds (*e.g.*
3 *Euplectes* widowbirds [58] and common yellowthroat *Geothlypis trichas* [59]), carotenoid-
4 containing colours in males are not always sexually attractive and may serve other purposes.

5

6

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Table 1. Animal species for which the mate-choice function of bright carotenoid-based colours in males has been tested. Only those studies where careful experimental work has been conducted are included.

| Vertebrate class | Species name | Common name | References |
|------------------|---------------------------------------|--------------------------|------------------|
| Osteichthyes | <i>Poecilia reticulata</i> | Guppy | [54,60,61,62,63] |
| | <i>Gasterosteus aculeatus</i> | Three-spined stickleback | [53,64] |
| Reptilia | <i>Sauromalus obesus</i> ^a | Chuckwalla | [58] |
| Aves | <i>Carpodacus mexicanus</i> | House finch | [65,66] |
| | <i>Carduelis tristis</i> | American goldfinch | [67] |
| | <i>Carduelis spinus</i> | Eurasian siskin | [68] |
| | <i>Malurus melanocephalus</i> | Red-backed fairy wren | [69] |
| | <i>Ploceus cucullatus</i> | Village weaverbird | [70] |
| | <i>Emberiza citrinella</i> | Yellowhammer | [71,72] |
| | <i>Taeniopygia guttata</i> | Zebra finch | [73,74] |

^aYellow/orange skin also derives some colour from pterin pigments.

Some female animals also display large, rich regions of carotenoid-based colour, in a few species matching or even surpassing those of males in colour and size. These female colours may be non-functional and simply genetically correlated with expression in males, especially when females display only vestiges of the male trait [75]. In several evolutionary lineages, however, female colour expression has become more elaborate [76,77], independent of any changes in male colour, which suggests an adaptive value. There is now good evidence that carotenoid-based colours in females of one fish species and three bird species catch the attention of males in mating contexts [78] (Table 2). For several other species, studies show assortative mating based on carotenoid-based colour (*i.e.* where more carotenoid-rich males tend to pair with more carotenoid-rich females, which has been interpreted by some as evidence for male mate choice), but such studies cannot rule out condition-dependent female mate choice or the role of intrasexual competition in pair-bond formation.

There is clear adaptive value to male mate choice in species where males invest heavily in offspring care, if carotenoid colouration is linked to the reproductive quality of females. Males do not always select the most colourful females, however (Table 2), and sometimes do not use colour at all in mate choice (*e.g.* red-winged blackbirds, *Agelaius phoeniceus*) [79].

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Table 2. Animal species in which female carotenoid-based colours are used by males in mate selection. Only species where direct mate choice was tested and confirmed with experimentation are included.

| Vertebrate class | Species name | Common name | References |
|------------------|-------------------------------|--------------------------|------------|
| Osteichthyes | <i>Gobiusculus flavescens</i> | Two-spotted goby | [80] |
| Aves | <i>Carpodacus mexicanus</i> | House finch ^a | [81] |
| | <i>Taeniopygia guttata</i> | Zebra finch ^b | [73] |
| | <i>Petronia petronia</i> | Rock petronia | [82] |

^a Only captive males exhibit this preference. In the wild, males pair with older females, who actually tend to be less colourful than young females.

^b Males prefer intermediately coloured females.

b) Social status signalling

i) Intra-sexual competition. Animals of the same sex often compete for limited or valuable space, food or mates and use signals that communicate their intent and ability to fight; this ensures that not all fights escalate to the point of injury or death [1]. Male birds that live in non-breeding foraging flocks frequently encounter new, unfamiliar rivals and could benefit from using signals that reveal their aggressive nature [83]. Carotenoid-containing colours are among the traits that serve as signals of social status in both fishes and birds [84]. The few examples where males with vibrant or larger colour patches have been shown to be dominant to males with smaller, less intense colours are listed in Table 3. This is not a universal role, however. In other species (*e.g.* house finch [85]; northern cardinal *Cardinalis cardinalis* [86]), there is no consistent link between male colour and aggression.

Females of many colourful species are also aggressive but, in the only example tested (the house finch), no relationship was found between plumage colouration and dominance [87].

Table 3. Animal species in which male carotenoid-based colours have been shown experimentally to serve as status signals.

| Vertebrate class | Species name | Common name | References |
|------------------|--------------------------------------|---------------------------------------|------------|
| Chondrichthyes | <i>Cichlasoma meeki</i> | Firemouth cichlid | [88] |
| Aves | <i>Euplectes ardens</i> | Red-collared widowbird ^a | [89,90] |
| | <i>Euplectes axillaris</i> | Red-shouldered widowbird ^a | [91,92] |
| | <i>Agelaius phoeniceus</i> | Red-winged blackbird ^a | [93,94] |
| | <i>Xanthocephalus xanthocephalus</i> | Yellow-headed blackbird | [95] |

^a These species also deposit melanin pigments into their colourful feather patches.

1 *ii) Individual recognition.* Signalling an individual's identity is common in animals that exhibit
2 discrete, genetically determined morphotypes and that live in crowded environments, where
3 otherwise it would be difficult to recognize neighbours and strangers [96]. Carotenoid-based
4 plumage is thought to serve this very function in the red-billed quelea (*Quelea quelea*) [97],
5 which is perhaps the most abundant bird on Earth and is found in densities of up to 60,000
6 birds per hectare.

7
8 c) Parent - offspring signalling

9
10 One of the classic examples of a behavioural releaser, *i.e.* a simple cue by one animal that
11 activates an instinctive response in another, is the carotenoid-based red beak spot of adult
12 herring gulls (*Larus argentatus*), which induces a chick to open its mouth to receive food [98].
13 Young birds also occasionally bear brilliant colours that serve to reveal to parents their
14 nutritional needs or their worth as offspring [99]. For example, the red mouth flush of begging
15 nestling common canaries (*Serinus canaria*) is directly reflects hunger [100]. In American coot
16 (*Fulicula americana*) chicks, orange head plumes attract parental attention; offspring lacking
17 them receive less food and grow more slowly [101]. Ephemeral mouth colours in young birds
18 are largely thought to be caused by increased flow of blood (red haemoglobin), whereas orange
19 colour in the feathers can be produced by carotenoid, phaeomelanin, or pterin pigments [102].
20 In one example, however, dietary supplementation with lutein (133) led to reddening of the
21 mouth of nestling barn swallows (*Hirundo rustica*) [103].

22
23 d) Other conspecific signal functions

24
25 Sexually variable traits can serve other conspecific-signalling functions [96] as (i) signals of
26 genetic relatedness or compatibility between prospective mates, (ii) signals of reproductive
27 strategy (*e.g.* sneaker *versus* territorial males), (iii) signals of presence (*e.g.* promoting group
28 cohesion), and (iv) amplifiers of other signals, to enhance detection and discrimination. To
29 date, there have been no studies of whether carotenoid-based colour in animals functions in
30 any of these ways.

31 Morphological traits such as colours may not serve only a single function, but may be used
32 for multiple purposes, *e.g.* signalling to both males and females, or be presented in conjunction
33 with other signals that reveal similar or different information [104]. For example, male
34 widowbirds use carotenoid-containing colour to signal social status to other males, but use tail
35 length to attract female mates [105]. No carotenoid-based ornament, however, has yet been
36 found to have dual utility in epigamic and intrasexual contexts (*e.g.* unlike melanic plumage
37 colouration in common yellowthroats [59]). It is also possible that carotenoid intake may

1 broadly influence the expression of many ornament types and serve as a ‘missing link’
2 underlying redundant signal expression in animals.

5 **D. Information Content of Carotenoid Signals**

7 Animal signals, including carotenoid-based colours, have the potential to reveal information
8 about nutritional state, disease status/health, genetic quality, aggressiveness, and fertility,
9 among other characteristics [1].

11 1. Nutritional state

13 Clearly, because carotenoids are derived from the diet, their acquisition from food is critical for
14 developing carotenoid-based colouration. Numerous studies of captive birds and fishes have
15 demonstrated that dietary deprivation of carotenoids reduces, and supplementation enhances,
16 carotenoid-based colour expression [51,106,107]. Typically, only colour intensity is affected
17 and not patch size, which is thought to be under stronger genetic control. Various
18 xanthophylls and carotenes can brighten the integument in colourful species [107], though
19 there are some exceptions; lycopene (**31**), violaxanthin (**259**), and β -carotene (**3**) have no effect
20 in common canaries [108], nor does β -carotene in American goldfinches (*Carduelis tristis*)
21 [107] or canthaxanthin (**380**) in carmine bee-eaters (*Merops nubicus*) [109]. In some species it
22 is clear that the ability to take up certain carotenoids from the diet is lacking (*e.g.* β -carotene
23 in green iguanas (*Iguana iguana*) [110]; lutein (**133**), zeaxanthin (**119**) and lycopene (**31**) in
24 American flamingos (*Phoenicopterus ruber*) [111]), though effects on colouration have not
25 been explored. In other species, notably the house finch, selectivity for colouration has been
26 identified; ingestion of common dietary xanthophylls (lutein and zeaxanthin) creates a yellow
27 plumage, whereas β -cryptoxanthin (**55**) is metabolized to ketocarotenoids such as 3’-
28 hydroxyechinenone (**295**) to make the feathers red [112,113].

29 But what nutritional factors are responsible for the colour variation seen in wild animals?
30 Does natural intake of carotenoids predict colour intensity and allow individuals to signal
31 nutritional proficiency with their colour signals? This appears to be the case in two species
32 that have been studied. Male guppies [114] and house finches [115] that had higher
33 concentrations of carotenoids in their gut contents exhibited deeper colours. Other studies
34 have accumulated consistent, though indirect, evidence that more colourful birds are superior
35 foragers, *e.g.* in Eurasian siskins (*Carduelis spinus*) [116]. Generally, however, little is known
36 about what carotenoids animals are ingesting in their food at the time that colour is being
37 developed.

1 There remains the question of whether general ‘nutritional state’ or total food intake
2 influences carotenoid-based colour intensity. The first study of this showed that male house
3 finches that grew feathers at a faster rate were more colourful [117]. By manipulating food
4 access, while controlling carotenoid intake, it was subsequently demonstrated that total caloric
5 intake influences plumage redness in male house finches [112]. This relationship was also
6 recently confirmed for female house finches [87] and for male American goldfinches
7 (*Carduelis tristis*) [118]. It is not known what controls this relationship. In American
8 goldfinches, the reduction in carotenoid levels was detected only in blood, so it was inferred
9 that carotenoid assimilation from food is sensitive to general nutrition [118]. Cholesterol plays
10 an important role in carotenoid accumulation and colouration in male zebra finches [119].

11 Recent studies of captive birds have revealed that the quality of nutrition received early in
12 life affects carotenoid assimilation at adulthood. Thus, poor early nutrition results in reduced
13 carotenoid colouration in adult ring-necked pheasants (*Phasianus colchicus*) [120], and in
14 reduced blood carotenoid levels in adult zebra finches [121]. Despite the reduced blood
15 carotenoid levels, zebra finches did not have reduced carotenoid pigmentation (bill redness)
16 and, in mate choice trials, were no less attractive to females than were control males [121].
17 Possibly, individuals of relatively short-lived species such as the zebra finch that have
18 received poor early nutrition may maximize sexual attractiveness to obtain a mate in the short
19 term, even if this may have adverse effects on health and potential lifespan [121].

20

21 2. Parasite load

22

23 Parasites and pathogens exert a broad range of effects on animal behaviour and physiology, so
24 it is not surprising that carotenoid-based colours are sensitive to disease status. Initial studies
25 supporting this link were done with poultry; ‘pale-bird syndrome’, *i.e.* the fading of yellow
26 tissues when chickens are infected with coccidian parasites, has long been documented [122].
27 The earliest empirical studies on the relationship between disease and colouration in wild
28 animals showed that, in two species of fish (three-spined sticklebacks and guppies),
29 parasitized males were less colourful and hence less preferred as mates by females [64,123].
30 The effects of different parasites (*e.g.* haematozoans, pox virus, coccidians, and ectoparasites
31 like fleas and ticks) on carotenoid pigmentation in birds have also been studied. Coccidians
32 have the strongest negative influence (*e.g.* in male and female house finches [87,124],
33 American goldfinches [125], and greenfinches, *Carduelis chloris* [126]), perhaps because they
34 inhabit the gut lining and directly disrupt nutrient absorption and lipoprotein production
35 [127], and drain internal carotenoid stores that may have a role in fighting infections [128].
36 Mycoplasmal conjunctivitis (caused by a bacterium) and avian pox (a virus) also reduce
37 carotenoid colouration in male house finches [129]. Macroparasites like mites and fleas act

1 similarly in a wide range of birds [130,131]. Haematozoans have less clear, consistent effects
2 on avian carotenoid pigmentation [106].

3 4 3. Immune defence

5
6 Brighter carotenoid colouration may signal an individual's greater capacity to defend against
7 parasites and diseases, and hence the greater ability to provide direct benefits such as parental
8 care [132]. The beneficial effects of carotenoids on the immune system are discussed
9 elsewhere (*Volume 5, Chapter 17*). Individuals with a lower burden of parasites or diseases
10 may have more carotenoids available, so that their superior health is advertised to prospective
11 mates [132]. This hypothesis is supported by numerous correlational studies [133], but there
12 has been limited experimental work. Dietary carotenoid supplementation of captive male
13 zebra finches confirmed that carotenoid availability can be limiting for sexual attractiveness
14 [74], and for cell-mediated and humoral immune responses following experimentally applied
15 challenge [74,134]. In European blackbirds (*Turdus merula*), inoculation with a novel antigen,
16 sheep red blood cells, resulted in diminished bill colouration, suggesting that humoral immune
17 activation depleted the body pool of carotenoids [135]. Similar findings have been reported for
18 zebra finches [134] and mallards [136].

19 Another means by which a relationship between immunocompetence and colouration can
20 be tested is to challenge animals that display their natural colour and examine whether more
21 colourful animals are more resistant to or can more quickly clear infections. In two species of
22 birds, more colourful individuals exhibited stronger responses to disease challenges.
23 Greenfinches that displayed larger yellow patches in tail feathers were better able to resist and
24 clear infection by Sindbis virus [137]. Redder male house finches cleared mycoplasmal
25 conjunctivitis infection faster [138]. A related study showed that vivid yellow leg colour is
26 strongly correlated with the ability of American kestrels (*Falco sparverius*) to counter blood-
27 parasite infections [139]. However, dietary carotenoid supplementation of captive male
28 American goldfinches had no effect on immune responses or disease resistance [140].

29 30 4. Antioxidant activity

31
32 It has been suggested that the expression of signals, including carotenoid colouration, may be
33 sensitive to an individual's oxidant-antioxidant balance, and ability to resist oxidative stress
34 [141]. Very few experimental studies have been performed, but one, with zebra finches, has
35 shown that carotenoid-supplemented individuals have greater resistance to oxidative damage,
36 supporting the hypothesis that more intense carotenoid colouration may advertise an
37 individual's superior capacity for antioxidant protection [142] (see *Volume 5, Chapter 12*).

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5. Fecundity

a) Egg production

Carotenoids are an important constituent of eggs, at least in taxa that produce yolky follicles [143]. If insufficient carotenoid is available, this may constrain a female's ability to lay, or the quality of her eggs. Studies of non-domesticated bird species in the wild and in captivity have shown that dietary supplementation with carotenoids can result in a range of beneficial effects including increased production of clutches [144], increased yolk carotenoid concentrations [145-147], reduced yolk susceptibility to lipid peroxidation [147,148], and increased offspring survival [147]. Similar effects have been observed in fish [149]. Females of many bird and fish species have carotenoid colouration, but it is not clear whether this may signal fecundity to males. In the two-spotted goby, a small marine fish, males prefer to mate with those females displaying the reddest abdomen [80], which reveals the pigmentation of the eggs visible through the abdominal wall [150]. There is little evidence, however, of a correlation between female colour (or egg carotenoid levels) and egg quality in this species [151]. In birds, house finch females with the most intense carotenoid plumage display are preferred as mates, but no association between female colouration and reproductive success was found [81]. In wild blue tits (*Parus caeruleus*) there was no relationship between the carotenoid-based plumage colouration of adult females and the degree of carotenoid deposition in eggs [145]. In contrast, both male and female captive zebra finches that were fed lutein and zeaxanthin developed a more colourful (carotenoid-based) beak which, in the female, directly correlated with the concentration of carotenoids delivered to the eggs [147]. However, female birds may invest more in offspring production (e.g. in egg carotenoids) when paired to a more attractive male [152].

b) Sperm production

It has been suggested that carotenoid colouration in males may be an indicator of fertility, i.e. the capacity to produce high quality sperm [153,154]. There is no evidence in the literature that carotenoids are themselves present in sperm or seminal plasma in any species bearing carotenoid colouration. However, if carotenoid colouration is a general indicator of overall health, this may include sperm quality.

Work with Trinidadian guppies has shown that carotenoid colouration predicts sperm reserves [155] and fertilizing success [156]. In mallards (*Anas platyrhynchos*), a link has been reported between bill colouration, higher blood levels of carotenoids and faster swimming

1 sperm [157]. Other studies, however, have found no correlation or even negative correlations
2 between carotenoid colouration and sperm quality parameters [154].

3 4 c) Offspring rearing

5
6 Studies of a range of bird species have suggested that individuals with greater carotenoid
7 colouration work harder at providing food for mates and nestlings [*e.g.* 66,158-160]. For
8 example, in a cross-fostering experiment with blue tits, chick growth was related to the
9 plumage yellowness of the foster father but not to that of the genetic parents [159].

10 11 6. Genetic quality

12
13 By mating with an elaborately ornamented male, a female may obtain ‘good genes’, *i.e.* genes
14 that aid in the survival and reproduction of her progeny. For this advantage to be conferred by
15 intense carotenoid-coloured males, colouration must be heritable and genetically coupled to
16 some measure of offspring quality. In one species (the zebra finch), quantitative genetic
17 studies show that carotenoid-based beak colour in males is highly heritable [161]. However,
18 this work was performed before it was known that maternal sources of yolk carotenoids could
19 confound studies of colour development in offspring. Future studies should be aimed at
20 determining whether carotenoid colouration and the fitness advantages it confers are linked to
21 candidate genes known to have fitness consequences in wild animals [162].

22 23 7. Photoprotection

24
25 Although it is visible to many animal taxa and provides important information during mate
26 choice and foraging [163], UV radiation has the potential to cause serious damage to
27 biomolecules. Most bird species moult once per year, at which time carotenoids are deposited
28 into growing feathers. Once feathers are fully grown, they are metabolically inert, so the
29 carotenoids contained in them are non-retrievable. Individuals in better health at the time of
30 moult may be able to allocate greater amounts of carotenoids to plumage display. Oxidation
31 caused by exposure to UV radiation could explain the seasonal fading of feather carotenoids
32 observed in green jays (*Cyanocorax yncas longirostris*) [164], great tits (*Parus major*) [165]
33 and house finches [166]. If so, the rate of bleaching of feather carotenoids could be inversely
34 related to the initial concentration of carotenoids deposited into feathers at the time of moult.
35 Feather colouration may, therefore, become progressively more reliable as an indicator of
36 individual condition over the course of a season, especially at the time that mate choice
37 decisions are made, which is typically several weeks or months post-moult. Bare parts (bill,

1 legs, *etc.*) are metabolically active, and could constitute a dynamic signal of defence against UV
2 exposure.

5 **E. Measurement of Carotenoids and Colour in Ecological Studies**

7 1. Carotenoid analysis and colour measurement

9 To provide the most information, accurate, reliable, detailed analysis of carotenoids is
10 essential. Thin-layer chromatography (TLC) is useful for screening, qualitative comparison
11 and isolation, but the method of choice for routine analysis is HPLC. All analyses must be
12 supported by the rigorous identification of the compounds involved. The principles and
13 practice of HPLC are described in *Volume 1A*, and the identification of carotenoids in *Volumes*
14 *1A* and *1B*. Recent procedures, guidelines and recommendations for HPLC and HPLC-MS are
15 given in *Volume 5, Chapter 2*.

16 HPLC is used routinely to analyse plants (leaves, fruit, flowers, roots *etc.*). With animals,
17 however, the technique can only be used to analyse museum and collected specimens,
18 pathological samples, blood and feathers. It cannot be used in the field. For information about
19 living animals and populations in the field, non-invasive methods for estimating carotenoids
20 have to be used.

21 In most ecological studies, carotenoid colouration is estimated qualitatively (*e.g.* by
22 categorizing individuals into ‘brighter’ and ‘duller’ groups), semi-quantitatively by colour
23 chips, or quantitatively by digital analysis of photographs or by reflectance
24 spectrophotometry [13,14,100-102]. Such non-invasive approaches do not give information
25 about the carotenoids and carotenoid profile [167]. Actual carotenoid profiles are known for
26 relatively few species (*e.g.* for less than 1% of the 8000 or more species of birds), and this
27 detailed analysis needs to be extended to a much wider range of animals, as a basis for
28 ecological studies. Once the relationship between colouration and carotenoid content has been
29 determined for a particular species, non-invasive colour measurements may then provide a
30 good indication of actual carotenoid levels in that species [167].

32 2. Sampling range

34 Most studies of the signal functions of carotenoid colouration have focused on vertebrates,
35 especially birds and fishes. Carotenoid colouration in signalling contexts has been remarkably
36 little studied in invertebrates, despite there being a considerable amount of detailed
37 information on the carotenoid profiles of these taxa [3]. Insects are notably under-represented

1 in the literature on carotenoid signalling, though this group accounts for over half of the 1.4
2 million species of animals that have so far been described [168]. There is, therefore, a need for
3 more studies of the signal functions of carotenoid colouration in taxa other than birds and
4 fishes.

5

6 3. Temporal changes in colouration

7

8 Studies of the information content of carotenoid colouration need to take into account the
9 potential for colour change. Bare parts such as skin may change colouration over relatively
10 short timescales, *e.g.* within a breeding season in male three-spined sticklebacks [169]. Even
11 feathers are not exempt from colour change, however. In birds, repeated measurements of
12 carotenoid colouration of plumage in the same individuals over successive years can show
13 remarkable variation [159]. Certain taxa may exhibit rapid and ephemeral colour changes as
14 stress responses, for example to capture or crowding, or in response to changes in physical
15 condition, motivation, or social status [170].

16

17

18 **F. Applied Value of Carotenoid Colouration**

19

20 1. Maintaining colouration and health in captive animals

21

22 The aesthetic value of carotenoid colour to humans cannot be measured scientifically, but its
23 financial value is clear to those involved in managing animals in the pet trade, in zoos, and for
24 commercial production. Pet owners and zoo visitors want to own or see attractively coloured,
25 healthy animals. Consumers want to purchase vibrant, fresh-looking meat and eggs.
26 Carotenoid-coloured species and products have long been a part of the pet trade (*e.g.*
27 canaries), zoo exhibits (*e.g.* flamingos), and produce markets (*e.g.* salmon steaks, chicken
28 meat/eggs), and decades of scientific research have been put into the need to maintain bright
29 colour in these and other domesticated animals [171-173]. Just as with the wild animals
30 described above, providing a carotenoid-rich diet to captive animals and housing them under
31 sufficiently healthy conditions is necessary to achieve optimal pigmentation.

32 Managers seeking pigmentation strategies for domestic animals can learn much from
33 evolutionary ecology. First and foremost, it should be determined whether or not the focal
34 trait or tissue in the captive species is carotenoid-based; not all red, orange, and yellow colours
35 are carotenoid-based (see [102] for review). Parrots, for example, do not use carotenoids to
36 develop such colours, but instead use a novel class of pigments (psittacofulvins [174,175])
37 whose production is not as sensitive to diet as are carotenoid colours.

1

2 2. Conservation implications of carotenoid signals

3

4 Animals use their carotenoid colours to communicate with one another about their quality and
5 viability, so there is every reason to believe that humans can similarly use these features in
6 'indicator species' to monitor attributes of population and environmental health *via* the
7 spatial and temporal variability that carotenoid colours exhibit [176,177]. Sexually selected
8 traits generally have this utility for being sensitive to, and hence revealing, recent
9 environmental perturbations and, for example, have been used to monitor effects of the
10 Chernobyl nuclear disaster in the 1980s [178]. To date, little empirical work has been done to
11 assess the conservation value of carotenoid colours, but several related observations
12 emphasize their potential in this respect. For example, the average carotenoid colouration in a
13 population of northern cardinals was found to decrease after a harsh winter that presumably
14 killed many fruit crops [179]. Moreover, a successfully re-introduced population of the
15 endangered hihi (*Notiomystis cincta*) from New Zealand was found to have the highest
16 carotenoid levels among all populations studied, suggesting that carotenoids were somehow
17 more available from the environment (or less demanded by the immune system) in these birds
18 [180]. Conservation plans are far too often adopted *after* significant mortality events or
19 environmental changes, perhaps because of the lack of easily collectable data that may point
20 to potential ecological or population declines. Because carotenoids are so closely linked to the
21 current environment and to an animal's current health state, they are good candidates for
22 background monitoring of the health and viability of animal populations and habitats. Samples
23 that can be collected relatively non-invasively, such as eggs or moulted feathers, may be
24 especially useful in this context.

25

26

27 **G. Conclusions**

28

29 The study of carotenoid colouration has grown apace over the past decade, as ecologists
30 increasingly embrace the tools of chemical analysis to glean insights into the information
31 content of carotenoid colouration. Investigations of epigamic traits in particular have been
32 fruitful in the development of theory, and improving our understanding of the information
33 content of carotenoid colouration. Yet in every area covered by this Chapter, unanswered
34 questions remain. For example, can carotenoid availability mediate crypsis? Can carotenoid
35 colouration in female animals signal their fecundity to prospective mates? Does carotenoid-
36 based gape colouration signal condition and neediness in chicks? Studies of taxa other than
37 birds and fishes, in particular insects, offer much potential for furthering our understanding of

1 carotenoid colouration. There is also a need for studies that bridge traditional ecological
2 research boundaries. For example do foragers target more carotenoid-rich flowers and fruits,
3 and does greater carotenoid allocation to signal expression ensure enhanced reproductive
4 success in plants? Are there costs to plants of allocating greater amounts of carotenoids to
5 colour signals as opposed to alternative somatic functions? Carotenoid colouration therefore
6 promises to remain a rich source of inspiration for new ideas and discovery, and there are great
7 opportunities for collaboration amongst behavioural ecologists, botanists, biochemists and
8 chemists.

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