

CrossMark
click for updates

Research

Cite this article: Higginson DM, Belloni V, Davis SN, Morrison ES, Andrews JE, Badyaev AV. 2016 Evolution of long-term coloration trends with biochemically unstable ingredients.

Proc. R. Soc. B **283**: 20160403.

<http://dx.doi.org/10.1098/rspb.2016.0403>

Received: 23 February 2016

Accepted: 21 April 2016

Subject Areas:

evolution, ecology

Keywords:

biochemical stability, carotenoids, evolutionary transitions, integration, moult

Author for correspondence:

Dawn M. Higginson

e-mail: dmhigginson@email.arizona.edu

[†]Present address: Center for Mind/Brain Sciences, University of Trento, Rovereto, Trentino, Italy.

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2016.0403> or via <http://rspb.royalsocietypublishing.org>.

Evolution of long-term coloration trends with biochemically unstable ingredients

Dawn M. Higginson, Virginia Belloni[†], Sarah N. Davis, Erin S. Morrison, John E. Andrews and Alexander V. Badyaev

Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ, USA

DMH, 0000-0003-4665-5902; VB, 0000-0001-9807-1912; ESM, 0000-0002-4487-6915

The evolutionarily persistent and widespread use of carotenoid pigments in animal coloration contrasts with their biochemical instability. Consequently, evolution of carotenoid-based displays should include mechanisms to accommodate or limit pigment degradation. In birds, this could involve two strategies: (i) evolution of a moult immediately prior to the mating season, enabling the use of particularly fast-degrading carotenoids and (ii) evolution of the ability to stabilize dietary carotenoids through metabolic modification or association with feather keratins. Here, we examine evolutionary lability and transitions between the two strategies across 126 species of birds. We report that species that express mostly unmodified, fast-degrading, carotenoids have pre-breeding moults, and a particularly short time between carotenoid deposition and the subsequent breeding season. Species that expressed mostly slow-degrading carotenoids in their plumage accomplished this through increased metabolic modification of dietary carotenoids, and the selective expression of these slow-degrading compounds. In these species, the timing of moult was not associated with carotenoid composition of plumage displays. Using repeated samples from individuals of one species, we found that metabolic modification of dietary carotenoids significantly slowed their degradation between moult and breeding season. Thus, the most complex and colourful ornamentation is likely the most biochemically stable in birds, and depends less on ecological factors, such as moult timing and migration tendency. We suggest that coevolution of metabolic modification, selective expression and biochemical stability of plumage carotenoids enables the use of unstable pigments in long-term evolutionary trends in plumage coloration.

1. Introduction

How do the contingent processes of development and function produce long-term evolutionary trends? Darwin considered this question to be central to his theory of evolution, because it would provide a crucial insight into the relationship between inheritance and natural selection [1], and between the developmental stability of complex phenotypes and their ability to accommodate and integrate novel inputs [2–4]. There are several insightful conceptual resolutions of this question [5–10], but empirical tests are rare because they require investigation of evolutionary trajectories where an environmentally contingent trait gets reliably incorporated into the phenotype and stabilized over evolutionary timescales.

One of the most striking examples of the recurrent incorporation and accommodation of environmentally contingent elements is the evolution of diet-dependent coloration in animals. For example in birds, complex and lineage-specific carotenoid-based ornamentation requires carotenoids obtained through the diet that are subsequently incorporated into the organismal phenotype [11]. Unbound carotenoids (and, in particular, dietary carotenoids) degrade rapidly with UV and oxygen exposure [12–14]. Thus, differences among carotenoids in biochemical stability, together with the empirical investigation of mechanisms by which birds stabilize and integrate these compounds

into their feathers, can provide important insight into the evolutionary integration of externally obtained elements into phenotypes.

Plumage-bound carotenoids are acquired through the diet during the short period of feather growth (moult), and can be either deposited directly or biochemically modified prior to deposition in the feather [15,16]. Once carotenoids become incorporated into the keratin matrix of a growing feather, no further addition of pigment is possible. Numerous observations of change in feather colour between subsequent moults showed that abrasion or other alterations of feather structure (e.g. by microbial activity, physical damage, UV exposure) progressively exposes embedded carotenoids to additional oxidation, and thus produces colour change despite the lack of additional pigment input [17–21]. An important discovery is that different carotenoid types within a feather have different potential for UV and oxygen modification, with some carotenoids remaining remarkably stable once deposited in feathers whereas others change readily, considerably modifying the appearance of the plumage [20–22]. The biochemical stability of carotenoids typically increases with metabolic modification [23,24], including those commonly accomplished by birds [25]. Thus, metabolic modification of carotenoids prior to deposition, the selective deposition of biochemically stable carotenoids, and the duration of post-deposition exposure prior to the breeding season (i.e. timing of moult) should coevolve.

Carotenoids can be *a priori* classified into degradation propensity groups based on their structural features (electronic supplementary material, appendix 1; [13,26]). For example, carotenoids with different structures vary in their potential for photo-oxidation, reactivity with free radicals, and in their associated tendency to produce carbonyl compounds and highly reactive epoxides [12,27–29]. Reduction in the number of conjugated double bonds in a carotenoid produces a shift towards lower wavelength absorbance, and corresponding changes in hue and intensity of the associated colour [14,30]. Differences between yellow and red carotenoids in their degradation propensity and photo-bleaching are consistent with the observed relative biochemical instability of yellow feather pigments compared to red and purple pigments (e.g. [31,32]).

To accommodate the biochemical instability of dietary carotenoids, birds could employ several strategies. Species could (i) evolve enzymatic pathways to convert these compounds to slower degrading forms prior to deposition in feathers, (ii) evolve compound-specific integration with feather proteins that minimizes carotenoid instability, (iii) selectively consume more slow-degrading dietary carotenoids or selectively express slow-degrading carotenoids or (iv) optimize the time between carotenoid acquisition (i.e. moult) and plumage display (i.e. breeding season). These strategies may be sequential evolutionary stages or alternative tactics pursued by different avian lineages. Optimizing the timing of carotenoid acquisition and display might involve the evolution of a pre-breeding moult for species that deposit mostly fast-degrading carotenoids. Alternatively, species might capitalize on the effects of feather abrasion, subsequent photo-oxidation and associated colour change to arrive at the most advantageous colour at the time of mating [17,19,33]. Species with only a post-breeding moult might have a greater prevalence of feather protective structures and modifications (e.g. unpigmented feather tips and barbules, denser keratin matrix) that enable pigment

preservation and optimal expression during the subsequent mating season.

Here, we examine coevolution and evolutionary lability of metabolic modification of consumed carotenoids, their expression in the plumage and timing of moult in 126 bird species. The timing of moult, its duration and intensity coevolves with migratory tendency in birds [33–36], which imposes an additional constraint on the acquisition, metabolism and subsequent duration of environmental exposure of the plumage carotenoids [37–40]. In turn, the juxtaposition of moult, breeding season and migration varies with geographical distribution and local seasonality. We statistically controlled for the effects of migration tendencies and geographical distribution on moult strategies and biochemical stability of plumage carotenoids. We also examine the mechanisms associated with evolutionary transitions from the expression of fast- to slow-degrading carotenoids in plumage and use repeated sampling of individuals of one species to directly examine the degradation propensities of feather carotenoids in relation to their metabolic modification.

2. Material and methods

(a) Data collection

Data on the timing of the nuptial moult in relation to the breeding season (here pre-breeding moult, post-breeding moult or both), migration tendency (migratory or resident—which includes altitudinal and short-range migrants), geographical distribution (North and Central America, South America, Eurasia, Africa and Oceania) and carotenoids present in the plumage for 126 bird species were collected from the literature (figure 1; electronic supplementary material, appendix 2). We also calculated the time (Δt , in days) from the midpoint of the breeding season to the midpoint of the closest moult period (figure 1). For species with both a pre- and post-breeding moult, Δt was calculated from the pre-breeding moult period. Data on metabolic pathway elongation (maximum number of enzymatic reactions from dietary to expressed carotenoids) and the selective expression of plumage carotenoids (proportion of carotenoid compounds expressed in the plumage relative to the total number of carotenoids in a species' metabolic network) are taken from appendices in [25,41] and are presented in figure 1.

(b) Categorization of carotenoid degradation propensity

Carotenoids were categorized as fast- or slow-degrading based on their chemical structure and the reactivity of their functional groups (electronic supplementary material, appendix 1). Carotenoids are characterized by a backbone of alternating double and single carbon–carbon bonds [42], which is subject to degradation by cleavage of the double bonds in the presence of oxygen, heat or light [29,43–45]. While carotenoids likely display a continuum of biochemical stability, discrete categories were required for our comparative analyses. Stability of the plumage carotenoids was calculated as the ratio of slow-degrading carotenoids to the total carotenoids present. For the subset of analyses using discrete traits (see below), plumage carotenoids were considered 'stable' if at least half of the expressed carotenoids were slow-degrading and 'unstable' if more than half of the expressed carotenoids were fast-degrading (electronic supplementary material, appendix 1).

(c) Empirical validation of degradation categories

To examine whether carotenoids that we classified as fast- and slow-degrading based on their structure (electronic supplementary

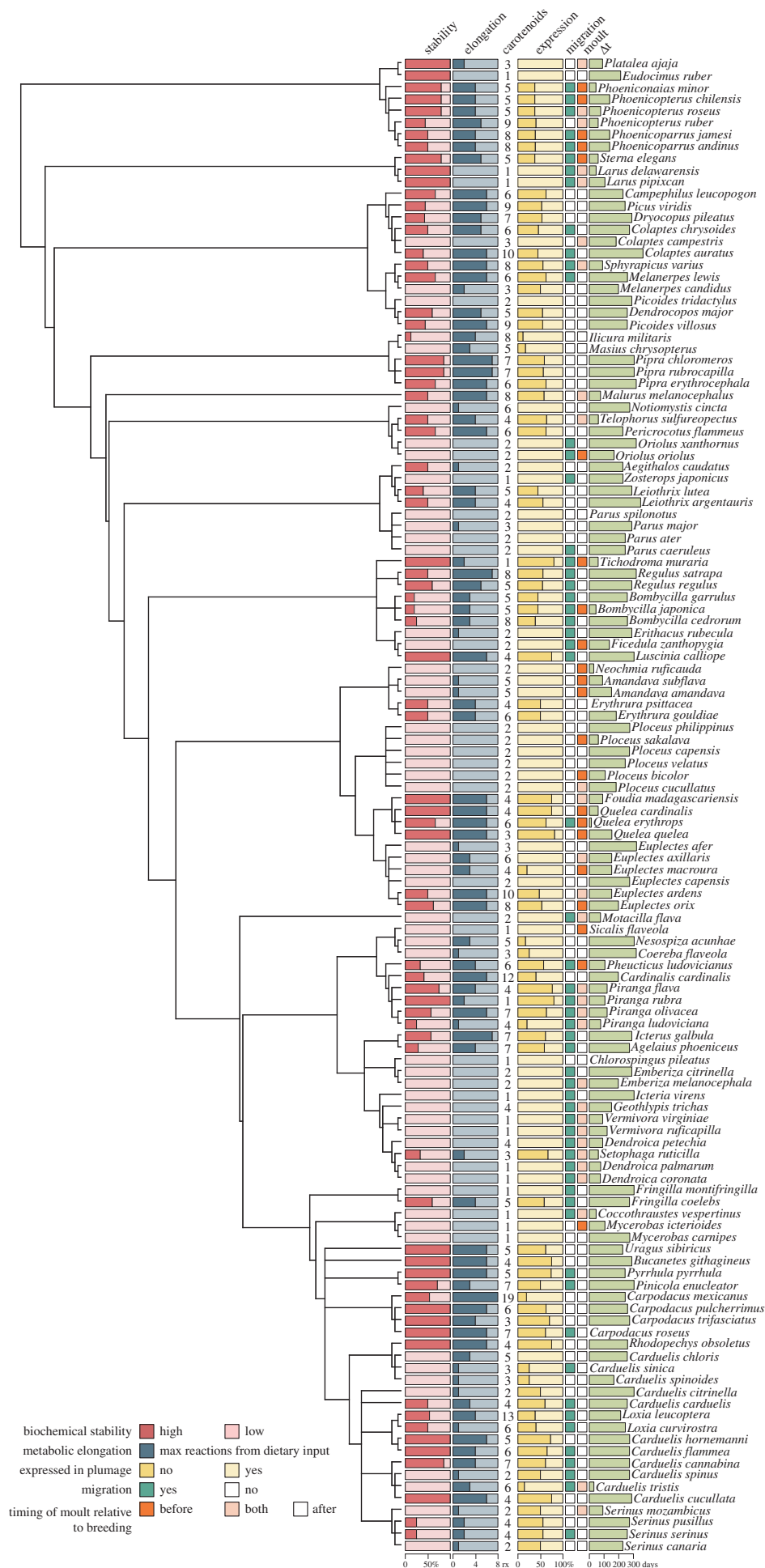


Figure 1. (Caption overleaf.)

Figure 1. (*Overleaf.*) Phylogenetic distribution of plumage carotenoid composition, moult type and migratory tendency shown on a strict consensus tree produced from a 1000 tree pseudo-posterior distribution. *Stability.* The percentage of fast- and slow-degrading carotenoids in the plumage. Stability can be achieved through biochemical modification of dietary carotenoids, consumption of slow-degrading carotenoids, or by the selective expression of slow-degrading carotenoids in the plumage. *Elongation.* The maximum number of enzymatic reactions between dietary and expressed carotenoids observed in a species (dark blue) relative to the maximum observed among the 126 species (length of the bar). *Carotenoids.* The total number of carotenoids expressed in the plumage. *Expression.* The percentage of non-expressed carotenoids (dietary compounds or metabolic intermediates, dark yellow) and those expressed in the plumage (light yellow). *Migration.* Species categorized as migratory (filled squares) include both long distance and partial migrants. Altitudinal and local migrants were classified as non-migratory (open squares). *Moult.* The timing of moult relative to breeding (dark orange denotes pre-breeding only; light orange, both pre- and post-breeding moults; white, post-breeding only). Δt : The mean time between moult and mating (no data were available for species without bars).

material, appendix 1) degrade at different rates under natural conditions, we repeatedly collected ornamental feathers from 54 adult male house finches (*Haemorrhous mexicanus*) in an individually colour-marked study population in southeastern Arizona (details in [38]). House finches are a particularly useful species to examine the validity of the degradation categories, because they express a similar number of slow- and fast-degrading carotenoids in their plumage [25,41]. For each male, we sampled three to five feathers from each ornamental area (breast, crown and rump) within 30 days of moult completion, and then again three to six months after the moult. The mean time between feather samples (mean \pm s.e.: 154 ± 6 days) approximately corresponds to the time between the species' single post-breeding moult (August–September) and breeding season (January–February) for this population [46]. See the electronic supplementary material, Methods for details of carotenoid extraction and identification.

(d) Bayesian analyses

To test for correlated evolution and to estimate evolutionary transition rates between moult timing, migration tendency, the stability of plumage carotenoids, elongation of metabolic pathways and selective expression of carotenoids in the plumage, we used reversible-jump Markov chain Monte Carlo (rj-MCMC [47–50]) and a 1000-tree sample from birdtree.org [51]. This method simultaneously accounts for phylogenetic uncertainty and visits evolutionary model and parameter combinations in proportion to their posterior probabilities given the trees, data and priors. See the electronic supplementary material Methods for details of the analyses.

(e) Multivariate linear models

We calculated independent linear contrasts on a majority rule consensus tree of the 1000 tree sample from [51] in MESQUITE v. 3.03 [52] using the PDAP v. 1.16 package [53]. To achieve normal distribution, the standardized contrasts were log- (Δt , selective expression, numbers of fast- and slow-degrading carotenoids) or arcsin- (metabolic elongation, ratio of slow/total carotenoids) transformed. We used general linear models in SAS v. 9.04 to calculate the least-square means for independent contrasts and no-intercept multiple regression coefficients.

3. Results

(a) Phylogenetic distribution of traits and correlates of carotenoid stability

The relationship between the stability of the plumage carotenoids, metabolic elongation, selective expression of carotenoids, migratory tendency and moult timing is shown in figure 1. Species in this study expressed 1–19 carotenoids in their plumage and had up to 24 carotenoids in their biochemical networks. Metabolic elongation and the number of fast- and slow-degrading plumage carotenoids covaried

with species' migratory tendency and geographical distribution (electronic supplementary material, table S1). Thus, in subsequent analyses we statistically controlled for migratory tendency and geographical distribution. Stability of the plumage carotenoids (ratio of slow-degrading to total carotenoids) was strongly correlated with the timing of moult, metabolic elongation of the enzymatic pathway from dietary to expressed carotenoids, and the selective expression of carotenoids (figure 2*a*). Species with greater metabolic elongation had higher stability of plumage carotenoids that was accomplished through both an increase in the ratio of slow-degrading carotenoids and by selective expression of these carotenoids in plumage (figure 3). We thus examined the rate and sequence of evolutionary transitions in the correlated expression of these parameters.

(b) Evolution of moult timing and carotenoids stability

The evolution of moult timing relative to breeding was strongly associated with the biochemical stability of the plumage carotenoids (log Bayes factor (BF) > 8.02) and with migratory tendency (log BF > 4.07). Moult timing was evolutionary labile, but some transitions were more likely than others (figure 4*a*): in species in this study, a pre-breeding moult evolved subsequently to a post-breeding moult and was less labile than the stability of the plumage carotenoids (figure 4*c*). Evolution of a pre-breeding moult was more likely when ancestral species were migratory. A single post-breeding moult typically evolved when ancestors were non-migratory (figure 4*b*).

(c) Mechanisms of plumage carotenoid stability

The stability of the plumage carotenoids was strongly correlated with metabolic elongation of enzymatic pathways and the selective expression of slow-degrading carotenoids (log BF > 17.55 and 15.66 , respectively). These two mechanisms were strongly positively correlated (log BF > 76.98), but metabolic elongation was more evolutionary labile than the selective expression of carotenoids (figure 4*d*). Greater biochemical stability of plumage carotenoids in species with longer metabolic pathways was due to the greater number of newly gained slow-degrading compounds (figure 3*d*) and their selective expression in plumage (figure 3*c*).

(d) Carotenoid degradation during the post-moult period

The relative concentration of carotenoids in house finch feathers changed during the post-moult period (figure 2*b*). The relative concentration of carotenoids *a priori* classified as 'fast-degrading' (electronic supplementary material, appendix 1; mostly dietary carotenoids) decreased $27.44 \pm 5.34\%$ (mean \pm s.e.), whereas

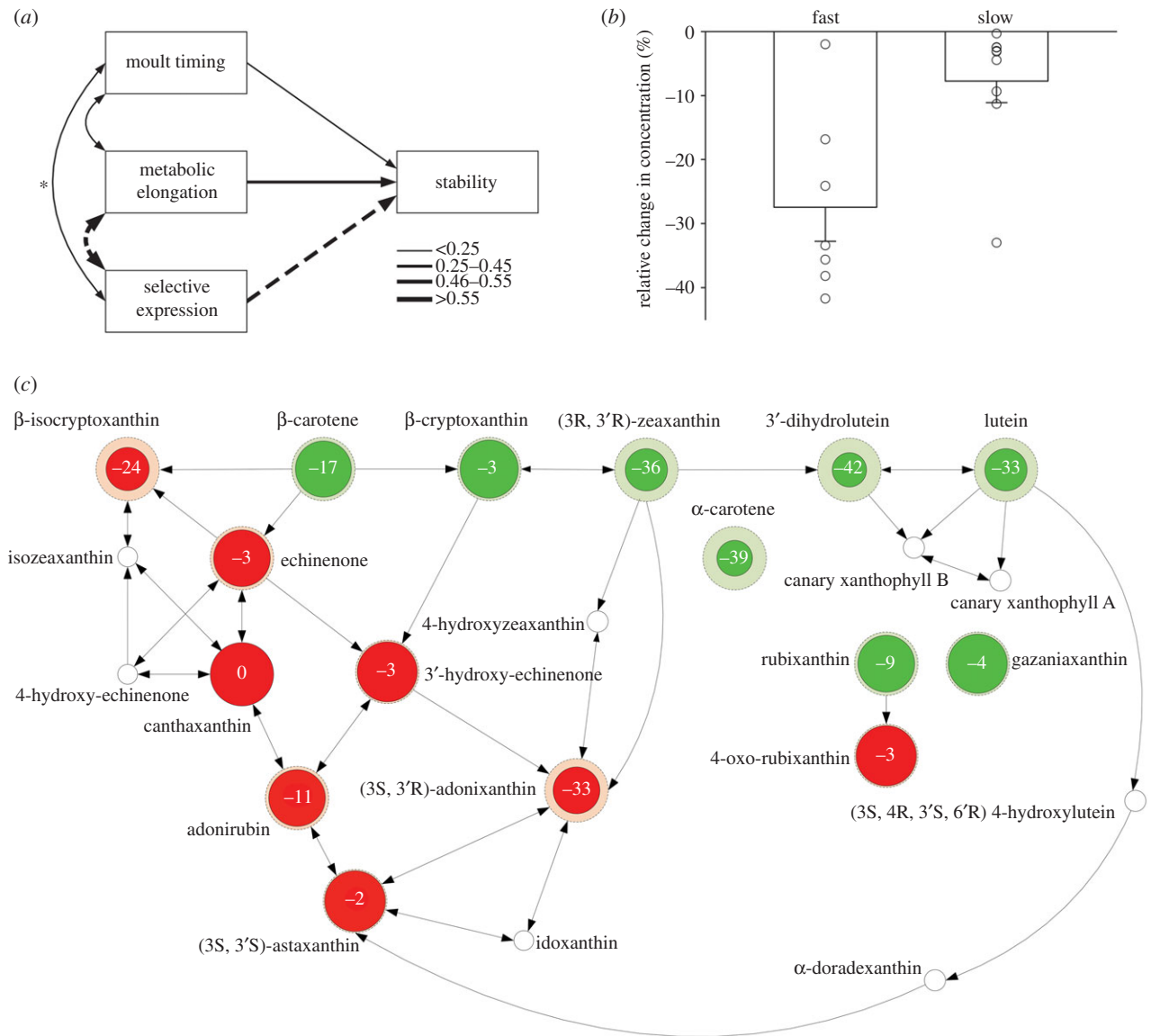


Figure 2. (a) Multiple regression of moult timing, metabolic elongation and selective expression of carotenoids on the stability of plumage carotenoids. Shown are the residuals of standardized independent linear contrasts after the effects of geographical distribution, phylogeny and migration tendency were statistically controlled. Double-headed arrows indicate covariation between the two variables. Single-headed arrows are regression coefficients. The solid lines indicate positive effects and dashed lines indicate negative effects. Line thickness indicates the magnitude of the effects (standardized regression coefficients, in standard deviations). Model-wide significance of the coefficients is based on a sequential Bonferroni test. Effects are significant at $\alpha < 0.01$ except where an asterisk indicates significance at $\alpha < 0.05$. (b) Relative change in plumage carotenoid concentration between subsequent samples (mean \pm s.e.) *a priori* designated as 'fast-' and 'slow-degrading' based on their structural characteristics. (c) Changes in the relative concentration of expressed carotenoids in fully grown feathers of male house finches 154 ± 6 days (mean \pm s.e., $n = 52$) after moult shown on the species metabolic network [25] that converts dietary carotenoids (shown in green) to modified carotenoids (shown in red) through enzymatic reactions (arrows). Shown are concentration changes (solid circle, second sample) in relation to concentration at moult completion (dashed circle, first sample). Small nodes are compounds not examined in this study. (Online version in colour.)

the concentration of those classified as 'slow-degrading' (mostly metabolically modified carotenoids) decreased $7.73 \pm 3.37\%$ between moult and the second sample 154 ± 6 days later (Kruskal–Wallis test $\chi^2 = 5.17$, $p = 0.02$; figure 2c).

4. Discussion

We found that the biochemical stability of plumage carotenoids increased with the length of the metabolic pathways and with the selective expression of slow-degrading, metabolically derived, carotenoids. Species that expressed primarily fast-degrading, metabolically unmodified, dietary carotenoids primarily fast-degrading, metabolically unmodified, dietary carotenoids in their plumage in their plumage had higher evolutionary lability of their moult timing. Among these species, an

additional, pre-breeding, moult was gained and lost readily and repeatedly from an ancestral state of only post-breeding moult (corroborating the results of [34,35]). Furthermore, the evolutionary lability of moult timing strongly covaried with migratory tendency (see also [33,54]): migratory species most often gained a pre-breeding moult, whereas non-migratory species most often lost it (figure 4). Conversely, species that expressed mostly slow-degrading carotenoids, as the result of metabolic modification or selective expression, had lower lability of their moult timing and a weaker association between the stability of plumage carotenoids and moult timing or migratory tendency.

Evolutionary association among moult timing, migration, metabolic modification conversion and the ability to selectively express carotenoids should be proportional to the consistency

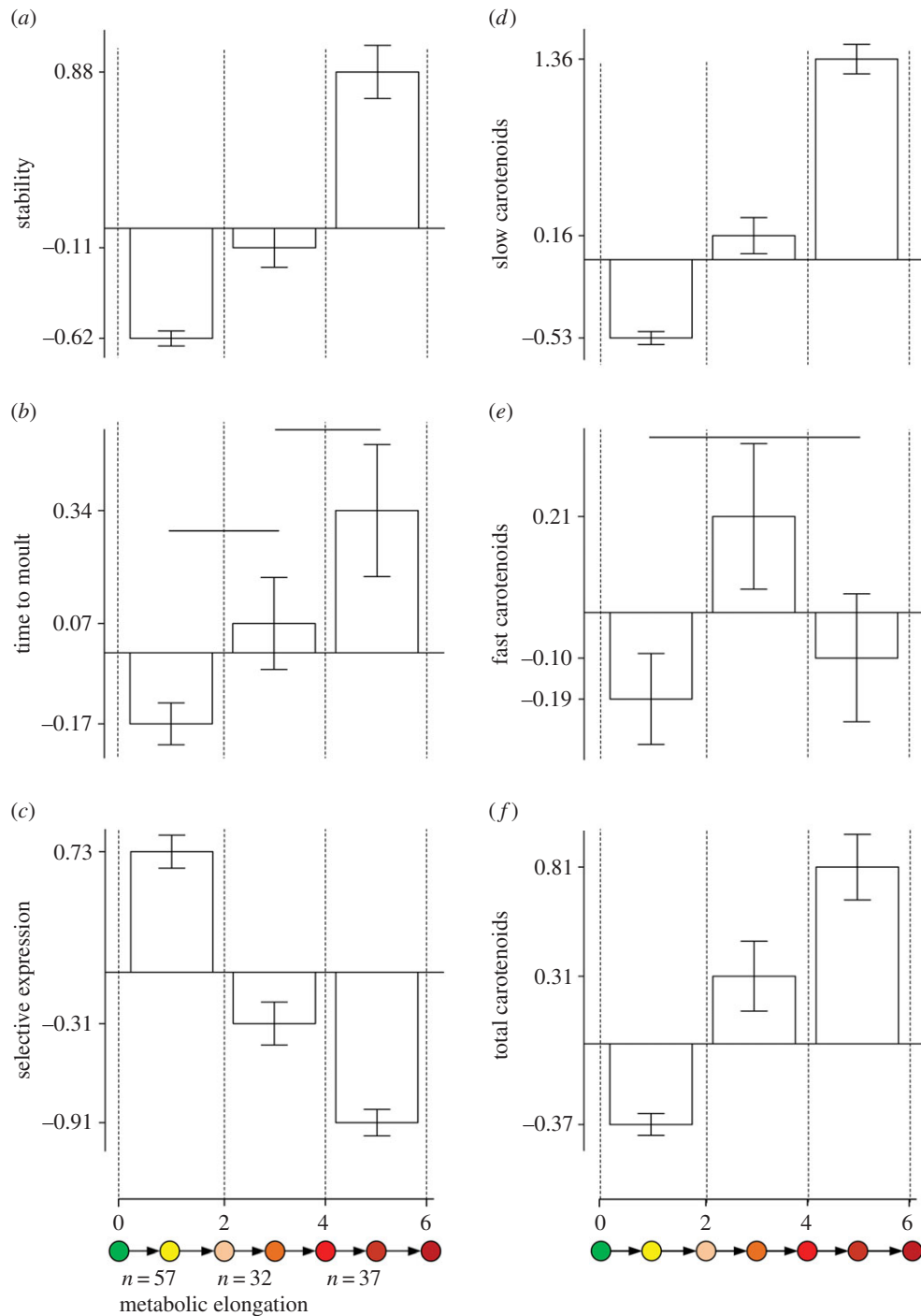


Figure 3. Variation in (a) the biochemical stability of plumage carotenoids (the ratio of slow-degrading carotenoids to the total carotenoids present), (b) time to moult (Δt), (c) selective expression, (d) number of slow-degrading carotenoid types, (e) number of fast-degrading carotenoid types and (f) total number of carotenoids, partitioned by metabolic elongation of the carotenoid pathway (longest distance from dietary to expressed carotenoid within a species, x -axis). Shown are least-squared means (\pm s.e.) for the residuals of independent linear contrasts after the effects of migration tendency and geographical distribution were statistically removed (electronic supplementary material, table S1). Metabolic elongation is divided into three groups (dashed lines; lower 33%, corresponding to distance of 0–2 enzymatic reactions from dietary compounds, 33–66%, corresponding to 3–4 enzymatic reactions and more than 66%, corresponding to more than 4 enzymatic reactions). Sample sizes (n) are shown as numbers of species in each category. Horizontal lines connect means that were not different between groups at $\alpha < 0.05$. (Online version in colour.)

of selection for such association. Lineages with access to a particular set of dietary carotenoids over evolutionary time might evolve metabolic pathways to convert these compounds to more slow-degrading forms and then selectively express the stabilized compounds. This would result in decreased interdependency between the stability of the plumage carotenoids and contemporary ecological factors (figures 2 and 4). At some stages of this process, such as when species start to use novel dietary compounds and tend to express them without modification, temporary gains of a pre-breeding moult might

facilitate use of these fast-degrading compounds in plumage displays. In turn, gain of an additional moult affects a suite of life-history traits, including migratory schedule, and could ultimately produce the frequently documented ecological associations among plumage displays, migration and range expansion [34,39,55–60].

Could elongate metabolic pathways that convert fast-degrading dietary carotenoids to slow-degrading carotenoids be a derived state within avian lineages, whereas direct and unselective expression of dietary carotenoids, be an ancestral

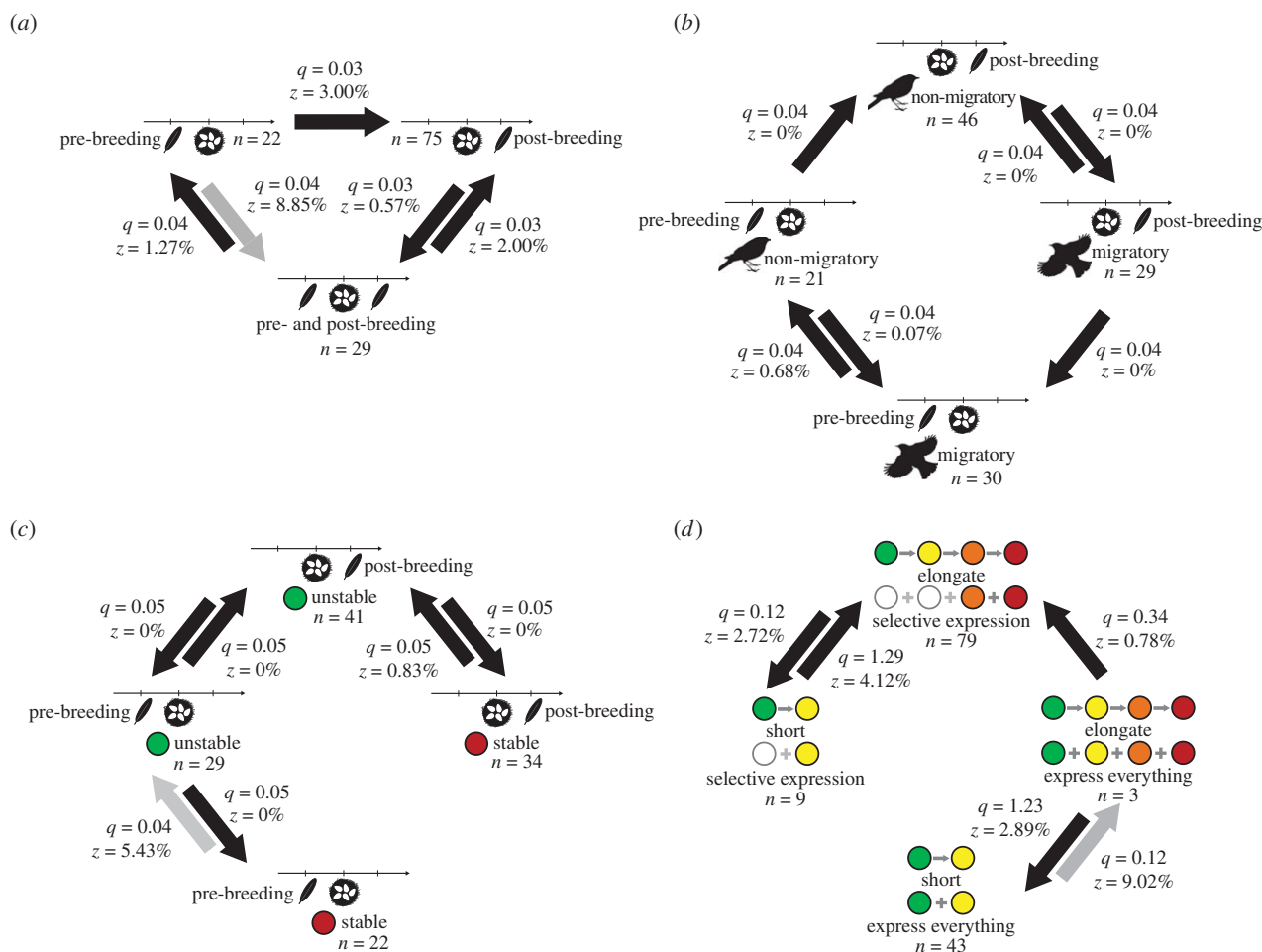


Figure 4. The rate and frequency of evolutionary transitions between the stability of plumage carotenoids, moult timing and migratory tendency given the tree sample, data and priors. Thick black arrows indicated probable evolutionary transitions (zero-value rate parameters in less than 5% of models visited by the rj-MCMC chain). Thick grey arrows indicate marginally likely evolutionary transitions (zero-value rate parameters in 5–11% of models). Absent arrows indicate improbable transitions (zero-value rate parameters in more than 19% of models) and n is the number of species. (a) Evolution of a single pre-breeding moult occurs only from ancestors with both a pre- and post-breeding moult, suggesting an evolutionary cycle in which a post-breeding moult is followed by a dual moult and then a single pre-breeding moult. Feathers indicate the timing of moult relative to breeding (indicated by a nest with eggs). (b) Evolutionary transitions from a post- to a pre-breeding moult was dependent on migratory tendency, with migratory species being more likely to gain a pre-breeding moult and non-migratory species more likely to lose a pre-breeding moult. (c) Evolution of moult timing was strongly correlated with the stability of plumage carotenoids: species with predominantly fast-degrading carotenoids (green circles) have more labile moult timing than species with predominantly slow-degrading carotenoids (red circles). (d) The mechanisms contributing to the stability of plumage carotenoids, elongation of metabolic pathways (i.e. increasing the number of modifications to dietary compounds) and selective expression of compounds (i.e. not expressing all compounds found in the diet or produced through metabolic modification), were very strongly correlated. Green circles represent fast-degrading, dietary carotenoids and the remaining circles are produced by metabolic modifications of dietary carotenoids, with red representing the slowest-degrading carotenoids. Open circles indicate unexpressed carotenoids. Metabolic pathways were considered short if there were less than or equal to one metabolic modification or elongate if greater than or equal to one modification. (Online version in colour.)

state? This scenario is unlikely because colour—the ultimate target of natural selection—is underlain by many factors, including metabolic conversion, feather keratin integration and post-deposition degradation of carotenoids, which interact to produce a multitude of potential evolutionary trajectories. For example, feather abrasion and the associated degradation of keratin-embedded carotenoids can produce a bright colour that is favoured during mating [17,19,33]. In such a case, a lineage might evolve moult timing or behavioural activities that enable the appropriate wear of protective feather structures and promote the oxidation of imbedded carotenoids. Once a favourable combination of dietary carotenoid stability, feather-wear and resulting colour is found, selection stabilizes and preserves this combination. However, because avian carotenoid pigmentation is dependent on the acquisition of dietary carotenoids the coevolution of expressed carotenoids, feather

keratins and other morphological or behavioural modifications is likely to ‘restart’ periodically when a lineage obtains novel dietary carotenoids. Indeed, on evolutionary timescales, elongation of carotenoid metabolic pathways was sustained by the periodic gain of new dietary compounds that connect to existing metabolic pathways [25]. This implies that the evolution of metabolic elongation is associated not only with the increased biochemical stability of derived carotenoids, but also with the recurrent inclusion of fast-degrading dietary compounds. Our results corroborated this scenario—the number of fast-degrading carotenoids did not differ among species with different metabolic elongation (figure 3e). Instead, the increased stability of plumage carotenoids in species with the longest metabolic pathways was caused by the selective expression of slow-degrading, derived carotenoids (figure 3c). Elongation of metabolic pathways and selective expression coevolved in

birds (figure 4). Evolution of selective expression likely accounts for the continuity of plumage colour and resulting plumage ornamentation trends despite the periodic addition of new fast-degrading dietary carotenoids during lineage evolution.

Connectivity of the enzymatic network that underlies carotenoid biosynthesis differs among avian clades and affects the patterns and rates of diversification in plumage carotenoids across species [25,41,61]. Our results suggest that a pre-breeding moult (and associated suite of ecological traits) should be especially prevalent in clades with limited opportunities for elongation of existing metabolic pathways or the addition of novel pathways. Similarly, a pre-breeding moult should be common in clades that consume a wide range of fast-degrading dietary carotenoids. Alternatively, these species might evolve protective feather structures that minimize carotenoid exposure and degradation (e.g. feather tips).

We found that selective expression of carotenoids (i.e. the ability not to express the intermediate metabolic stages) evolves readily in birds (figures 1, 2 and 4), although it had lesser evolutionary lability than metabolic elongation. Several carotenoids are usually present in developing feather follicles and there is evidence that their pattern of selective absorption is determined by the sequence in which carotenoid precursors and products are delivered to the follicle, competition for space or binding sites in feather keratin matrix, differences among carotenoids in their effect on feather structure, or an evolved specificity of feather keratin–carotenoid associations [62–65]. The extent to which these mechanisms are evolutionary steps or alternative trajectories in the evolution of selective expression of carotenoids in the plumage is an open question. Some species produce their plumage coloration by consuming and expressing an array of dietary precursors, intermediates and end products of carotenoid metabolism, whereas others express only the end products (e.g. [66,67]). It is likely that selective expression involves the evolution of specificity in feather keratin–carotenoid associations and the selective permeability of feather follicles to certain carotenoids. Within a species, the presence of carotenoid compounds in a follicle was associated with early structural differentiation of the growing feather [38]. Feather growth rate, and associated moult duration, was highly variable among individuals, but this did not covary with the type or concentration of incorporated carotenoids [68]. This suggests that early organizational effects of carotenoids on feather development and structure play an important role

in selective uptake and expression of carotenoids. The selective expression of carotenoids is routinely involved in modulating plumage polymorphism (species-, age-, sex- or season-related, [69]), such that the rapid evolution of selective expression likely involves cooption of existing mechanisms for the selective association of pigment with the integument.

In sum, the likely coevolution of metabolic pathway elongation and selective expression of carotenoids might enable long-term evolutionary trends of colourful plumage despite the chemical instability of carotenoids that colour it. Considering metabolic, ecological and life-history traits in the same phylogenetic framework shows that external ecological factors, such as timing of moult in relation to breeding and migration schedules, might be most important in the early stages of plumage ornamentation evolution within lineages. Subsequent coevolution of metabolic pathway elongation with the selective expression of derived, slow-degrading carotenoids results in greater organismal integration of colourful displays and their decreased dependency on current ecological factors.

Ethics. All procedures involving birds were approved by University of Arizona's Animal Care committee IACUC 13-423.

Data accessibility. Data and phylogenetic trees used in this study are available in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.8s2p3>.

Authors' contributions. A.V.B. designed the study and supervised field and laboratory work. V.B., E.S.M., A.V.B. and S.N.D. constructed the species metabolic networks and prepared the datasets. V.B., E.S.M., S.N.D. and J.E.A. analysed biochemical data. D.M.H. derived the analytical approach. D.H.M. and A.V.B. performed the analyses and wrote the paper with input from V.B. and E.S.M. All authors discussed the results and approved the manuscript.

Competing interests. The authors have no competing interests.

Funding. This research was supported by the grants from National Science Foundation (DEB-0077804 and IBN-0218313) and by the David and Lucile Packard Foundation Fellowship to A.V.B., and by the National Institute of General Medical Sciences of the National Institutes of Health Award (K12GM000708) to D.M.H. S.N.D. and J.E.A. were supported by REU supplements to the NSF awards and by UBRP programme at the University of Arizona.

Acknowledgements. Discussions with Renée Duckworth and Georgy Semenov provided useful insights. We thank the Editor, Associate Editor and anonymous reviewers for comments on earlier versions of this paper.

Disclaimer. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

1. Darwin C. 1859 *The origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. London, UK: John Murray.
2. Baldwin JM. 1902 *Development and evolution*. New York, NY: Macmillan.
3. Schmalhausen II. 1938 *Organism as a whole in individual development and history*. Leningrad: Academy of Sciences, USSR.
4. Badyaev AV. 2011 Origin of the fittest: link between emergent variation and evolutionary change as a critical question in evolutionary biology. *Proc. R. Soc. B* **278**, 1921–1929. (doi:10.1098/rspb.2011.0548)
5. West-Eberhard MJ. 2003 *Developmental plasticity and evolution*. Oxford, UK: Oxford University Press.
6. Müller GB, Newman S. 2003 *Origination of organismal form: beyond the gene in developmental and evolutionary biology*. Cambridge, MA: MIT Press.
7. Reid RGB. 2007 *Biological emergences: evolution by natural experiment*. Cambridge, MA: MIT Press.
8. Kirschner M, Gerhart JC. 2005 *The plausibility of life: resolving Darwin's dilemma*. New Haven, CT: Yale University Press.
9. Wagner A. 2011 *The origins of evolutionary innovations: a theory of transformative change in living systems*. New York, NY: Oxford University Press.
10. Laland KN, Uller T, Feldman MW, Sterelny K, Müller GB, Moczek A, Jablonka E, Odling-Smee J. 2015 The extended evolutionary synthesis: its structure, assumptions and predictions. *Proc. R. Soc. B* **282**, 20151019. (doi:10.1098/rspb.2015.1019)
11. Goodwin TW. 1984 *Biochemistry of the carotenoids: animals v. 2*. 2nd edn. London, UK: Chapman and Hall.

12. Boona CS, McClements DJ, Weissa J. 2010 Factors influencing the chemical stability of carotenoids in foods. *Crit. Rev. Food Sci.* **50**, 515–532. (doi:10.1080/10408390802565889)
13. Woodall AA, Lee SWM, Weesie RJ, Jackson MJ, Britton G. 1997 Oxidation of carotenoids by free radicals: relationship between structure and reactivity. *Biochim. Biophys. Acta-Gen. Subjects* **1336**, 33–42. (doi:10.1016/S0304-4165(97)00006-8)
14. Mortensen A, Skibsted LH, Lester P. 1999 Carotenoid photobleaching. *Method. Enzymol.* **299**, 408–421. (doi:10.1016/S0076-6879(99)99039-0)
15. McGraw KJ. 2006 The mechanics of carotenoid coloration in birds. In *Bird coloration. I. Mechanisms and measurements* (eds GE Hill, KJ McGraw), pp. 177–242. Cambridge, MA: Harvard University Press.
16. Brush AH. 1990 Metabolism of carotenoid pigments in birds. *FASEB J.* **4**, 2969–2977.
17. Blanco G, Frias O, Garrido-Fernandez J, Hornero-Mendez D. 2005 Environmental-induced acquisition of nuptial plumage expression: a role of denaturation of feather carotenoproteins? *Proc. R. Soc. B* **272**, 1893–1900. (doi:10.1098/rspb.2005.3157)
18. Willoughby EJ, Murphy M, Gorton HL. 2002 Molt, plumage abrasion, and color change in Lawrence's goldfinch. *Wilson Bull.* **114**, 380–392. (doi:10.1676/0043-5643(2002)114[0380:MPAAC]2.0.CO;2)
19. Troy DM, Brush AH. 1983 Pigments and feather structure of the redpolls, *Carduelis flammaea* and *C. hornemanni*. *Condor* **85**, 443–446. (doi:10.2307/1367983)
20. Delhey K, Burger C, Fiedler W, Peters A. 2010 Seasonal changes in colour: a comparison of structural, melanin- and carotenoid-based plumage colours. *PLoS ONE* **5**, e11582. (doi:10.1371/journal.pone.0011582)
21. McGraw KJ, Hill GE. 2004 Plumage color as a dynamic trait: carotenoid pigmentation of male house finches (*Carpodacus mexicanus*) fades during the breeding season. *Can. J. Zool.* **82**, 734–738. (doi:10.1139/Z04-043)
22. Stradi R, Celentano G, Boles M, Mercato F. 1997 Carotenoids in bird plumage: the pattern in a series of red-pigmented Carduelinae. *Comp. Biochem. Phys. B* **117**, 85–91. (doi:10.1016/S0305-0491(96)00271-4)
23. Schmidt-Dannert C, Umeno D, Arnold FH. 2000 Molecular breeding of carotenoid biosynthetic pathways. *Nat. Biotechnol.* **18**, 750–753. (doi:10.1038/77319)
24. Umeno D, Tobias AV, Arnold FH. 2005 Diversifying carotenoid biosynthetic pathways by directed evolution. *Microbiol. Mol. Biol. Rev.* **69**, 51–78. (doi:10.1128/MMBR.69.1.51-78.2005)
25. Badyaev AV, Morrison ES, Belloni V, Sanderson MJ. 2015 Tradeoff between robustness and elaboration in carotenoid networks produces cycles of avian color diversification. *Biol. Direct* **10**, 45. (doi:10.1186/s13062-015-0073-6)
26. Jorgensen K, Skibsted LH. 1993 Carotenoid scavenging of radicals. Effect of carotenoid structure and oxygen partial pressure on antioxidative activity. *Z. Lebensm. Unters. Forsch.* **196**, 423–429. (doi:10.1007/BF01190806)
27. Miller NJ, Sampson J, Candeias LP, Bramley PM, Rice-Evans CA. 1996 Antioxidant activities of carotenes and xanthophylls. *FEBS Lett.* **384**, 240–242. (doi:10.1016/0014-5793(96)00323-7)
28. Di Mascio P, Devasagayam TP, Kaiser S, Sies H. 1990 Carotenoids, tocopherols and thiols as biological singlet molecular oxygen quenchers. *Biochem. Soc. T.* **18**, 1054–1056. (doi:10.1042/bst0181054)
29. Mordí RC. 1993 Mechanism of beta-carotene degradation. *Biochem. J.* **292**, 310–312. (doi:10.1042/bj2920310)
30. Meléndez-Martínez AJ, Britton G, Vicario IM, Heredia FJ. 2007 Relationship between the colour and the chemical structure of carotenoid pigments. *Food Chem.* **101**, 1145–1150. (doi:10.1016/j.foodchem.2006.03.015)
31. Test FH. 1940 Effects of natural abrasion and oxidation on the coloration of flickers. *Condor* **42**, 76–80. (doi:10.2307/1364322)
32. McNett GD, Marchetti K. 2005 Ultraviolet degradation in carotenoid patches: live versus museum specimens of wood warblers (Parulidae). *Auk* **122**, 793–802. (doi:10.1642/0004-8038(2005)122[0793:Udicpl]2.0.CO;2)
33. Tökölyi J, Bókonyi V, Barta Z. 2008 Seasonal colour change by moult or by the abrasion of feather tips: a comparative study. *Biol. J. Linn. Soc.* **94**, 711–721. (doi:10.1111/j.1095-8312.2008.01027.x)
34. de la Hera I, Perez-Tris J, Telleria JL. 2006 Migratory behaviour affects the trade-off between feather growth rate and feather quality in a passerine bird. *Biol. J. Linn. Soc.* **97**, 98–105. (doi:10.1111/j.1095-8312.2008.01189.x)
35. Svensson E, Hedenstrom A. 1999 A phylogenetic analysis of the evolution of moult strategies in Western Palearctic warblers (Aves: Sylviidae). *Biol. J. Linn. Soc.* **67**, 263–276. (doi:10.1111/j.1095-8312.1999.tb01864.x)
36. Dawson A. 2008 Control of the annual cycle in birds: endocrine constraints and plasticity in response to ecological variability. *Phil. Trans. R. Soc. B* **363**, 1621–1633. (doi:10.1098/rstb.2007.0004)
37. Serra L, Griggio M, Licheri D, Pilastro A. 2007 Molt speed constrains the expression of a carotenoid-based sexual ornament. *J. Evol. Biol.* **20**, 2028–2034. (doi:10.1111/j.1420-9101.2007.01360.x)
38. Landeen EA, Badyaev AV. 2012 Developmental integration of feather growth and pigmentation and its implications for the evolution of diet-derived coloration. *J. Exp. Zool. B Mol. Dev. Evol.* **318**, 59–70. (doi:10.1002/jez.b.21445)
39. Fitzpatrick S. 1994 Colorful migratory birds: evidence for a mechanism other than parasite resistance for the maintenance of good genes sexual selection. *Proc. R. Soc. Lond. B* **257**, 155–160. (doi:10.1098/rspb.1994.0109)
40. Norris DR, Marra PP, Montgomerie R, Kyser TK, Ratcliffe LM. 2004 Reproductive effort, molting latitude, and feather color in a migratory songbird. *Science* **306**, 2249–2250. (doi:10.1126/science.1103542)
41. Morrison ES, Badyaev AV. In review. Structuring evolution: biochemical networks and color diversification in birds.
42. Britton G, Llaaen-Jensen S, Pfander H. 2004 *Carotenoid handbook*. Basel, Switzerland: Birkhäuser.
43. Etoh H, Suhara M, Tokuyama S, Kato H, Nakahigashi R, Maejima Y, Ishikura M, Terada Y, Maoka T. 2012 Auto-oxidation products of astaxanthin. *J. Oleo Sci.* **61**, 17–21. (doi:10.5650/jos.61.17)
44. Aparicio-Ruiz R, Mínguez-Mosquera MI, Gandul-Rojas B. 2011 Thermal degradation kinetics of lutein, β -carotene and β -cryptoxanthin in virgin olive oils. *J. Food Comp. Anal.* **24**, 811–820. (doi:10.1016/j.jfca.2011.04.009)
45. Scita G. 1992 Stability of beta-carotene under different laboratory conditions. *Methods Enzymol.* **213**, 175–185. (doi:10.1016/0076-6879(92)13120-M)
46. Badyaev AV, Belloni V, Hill GE. 2012 House finch (*Haemorhous mexicanus*). In *The birds of North America online* (ed. A Poole). Ithaca, NY: Cornell Lab of Ornithology. See <http://bna.birds.cornell.edu.bnaproxy.birds.cornell.edu/bna/species/046>. (doi:10.2173/bna.46)
47. Meade A, Pagel M. 2014 *BayesTraits*, version 2. See <http://www.evolution.rdg.ac.uk/BayesTraits.html>.
48. Pagel M. 1994 Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proc. R. Soc. Lond. B* **255**, 37–45. (doi:10.1098/rspb.1994.0006)
49. Pagel M, Meade A. 2006 Bayesian analysis of correlated evolution of discrete characters by reversible-jump Markov chain Monte Carlo. *Am. Nat.* **167**, 808–825. (doi:10.1086/503444)
50. Pagel M, Meade A, Barker D. 2004 Bayesian estimation of ancestral character states on phylogenies. *Syst. Biol.* **53**, 673–684. (doi:10.1080/10635150490522232)
51. Jetz W, Thomas GH, Joy JB, Hartmann K, Mooers AO. 2012 The global diversity of birds in space and time. *Nature* **491**, 444–448. (doi:10.1038/nature11631)
52. Maddison WP, Maddison DR. 2015 *Mesquite: a modular system for evolutionary analysis*, version 3.03. See <http://mesquiteproject.wikispaces.com/home>.
53. Midford PE, Garland Jr T, Maddison WP. 2011 *PDAP package of mesquite*, version 1.16. See http://mesquiteproject.org/pdap_mesquite/index.html.
54. Figuerola J, Jovani R. 2001 Ecological correlates in the evolution of moult strategies in Western Palearctic passerines. *Evol. Ecol.* **15**, 183–192. (doi:10.1023/A:1014824700389)
55. Chui CKS, McGraw KJ, Doucet SM. 2011 Carotenoid-based plumage coloration in golden-crowned kinglets *Regulus satrapa*: pigment characterization and relationships with migratory timing and condition. *J. Avian Biol.* **42**, 309–322. (doi:10.1111/j.1600-048X.2011.05240.x)
56. Badyaev AV, Ghalambor CK. 1998 Does a trade-off exist between sexual ornamentation and ecological plasticity? Sexual dichromatism and occupied elevational range in finches. *Oikos* **82**, 319–324. (doi:10.2307/3546972)

57. McLain DK. 1993 Cope's rules sexual selection and the loss of ecological plasticity. *Oikos* **68**, 490–500. (doi:10.2307/3544917)
58. McLain DK, Moulton MP, Redfearn TP. 1995 Sexual selection and the risk of extinction of introduced birds on oceanic islands. *Oikos* **74**, 27–34. (doi:10.2307/3545671)
59. Norris DR, Marra PP, Kyser TK, Ratcliffe LM, Montgomerie R. 2007 Continent-wide variation in feather colour of a migratory songbird in relation to body condition and moulting locality. *Biol. Lett.* **3**, 16–19. (doi:10.1098/rsbl.2006.0572)
60. Badyaev AV. 1997 Altitudinal variation in sexual dimorphism: a new pattern and alternative hypotheses. *Behav. Ecol.* **8**, 675–690. (doi:10.1093/beheco/8.6.675)
61. Morrison ES, Badyaev AV. In press. The landscape of evolution: reconciling structural and dynamic properties of metabolic networks in adaptive diversifications. *Integr. Comp. Biol.* (doi:10.1093/icb/icw026)
62. McGraw KJ, Beebe MD, Hill GE, Parker RS. 2003 Lutein-based plumage coloration in songbirds is a consequence of selective pigment incorporation into feathers. *Comp. Biochem. Phys. B* **135**, 689–696. (doi:10.1016/S1096-4959(03)00164-7)
63. Mendes-Pinto MM, LaFountain AM, Stoddard MC, Prum RO, Frank HA, Robert B. 2012 Variation in carotenoid–protein interaction in bird feathers produces novel plumage coloration. *J. R. Soc. Interface* **9**, 3338–3350. (doi:10.1098/rsif.2012.0471)
64. Völker O. 1934 Die abhängigkeit der lipochrombildung bei vögeln von pflanzlichen carotinoiden. *J. Ornithol.* **82**, 439–440. (doi:10.1007/bf01905418)
65. Stradi R, Celentano G, Rossi E, Rovati G, Pastore M. 1995 Carotenoids in bird plumage: I. The carotenoid pattern in a series of Palearctic Carduelinae. *Comp. Biochem. Phys. B* **110**, 131–143. (doi:10.1016/0305-0491(94)00136-1)
66. Prum RO, LaFountain AM, Berro J, Stoddard MC, Frank HA. 2012 Molecular diversity, metabolic transformation, and evolution of carotenoid feather pigments in cotingas (Aves: Cotingidae). *J. Comp. Physiol. B* **182**, 1095–1116. (doi:10.1007/s00360-012-0677-4)
67. Inouye CY, Hill GE, Stradi RD, Montgomerie R. 2001 Carotenoid pigments in male house finch plumage in relation to age, subspecies, and ornamental coloration. *Auk* **118**, 900–915. (doi:10.1642/0004-8038(2001)118[0900:CPIMHF]2.0.CO;2)
68. Badyaev AV, Vleck CM. 2007 Context-dependent ontogeny of sexual ornamentation: implications for a trade-off between current and future breeding efforts. *J. Evol. Biol.* **20**, 1277–1287. (doi:10.1093/icb/icm058)
69. Badyaev AV. 2007 Evolvability and robustness in color displays: bridging the gap between theory and data. *Evol. Biol.* **34**, 61–71. (doi:10.1007/s11692-007-9004-5)

Supplementary Methods

Carotenoid extraction and identification-- Feather carotenoids were extracted using high-performance liquid chromatography (HPLC) and feathers from different ornamental areas were processed separately. Briefly, feathers were trimmed, and the weighed pigmented portions were washed in hexane using Whatman GF/A glass filters and finely ground in 3mL methanol for 10 min at 20Hz using a Retsch MM301 mixer mill (Newtown, PA), equipped with ZrO grinding jars and balls. Carotenoids were extracted using a 0.2 μ m filter (GHP Arcodisc 13mm Minispikes; Pall Life Sciences, East Hills, NY), and the filtrate was dried under vacuum at 40C and reconstituted in 150 μ L of HPLC mobile phase (methanol:acetonitrile 50:50, v/v). Carotenoids were quantified by injecting 50 μ L of pigment extract into an HPLC System (Shimadzu Corporation, Pleasanton, CA) fitted with an YMC Carotenoid 5.0 μ m column (250x4.6mm) and guard column (YMC America, Allentown, PA). Analytes were eluted at a constant flow rate of 1.1mL/min using isocratic elution with 42:42:16 (v/v/v) methanol:acetonitrile:dichloromethane for the first 11 min, followed by linear gradient up to 42:23:35 (v/v/v) methanol:acetonitrile:dichloromethane through 21 min, isocratic elution at this condition until 30 min when it returned with step function to the initial isocratic condition at which it was held through 40 min. Carotenoids were detected using a Shimadzu SPD-M10AVP photodiode array detector, and data were collected from 200 to 800 nm. Peak areas were integrated at 450 or 470 nm depending on the absorbance maximum (λ max) for each compound. Peaks were identified by comparison with the retention times of standard carotenoid compounds (Sigma-Aldrich, St. Louis, MO; Indofine Chemical, Hillsborough, NJ; CaroteNature, Ostermundigen, Switzerland; Santa Cruz Biotechnology, Dallas, TX) and the concentrations of compounds (μ g/g) were calculated using calibration curves of these standards. We then converted these concentrations into percentages of total amount of carotenoids for each sample interval and obtained changes in relative concentration between the subsequent samples of the same individuals. Changes in relative concentration were averaged across ornamental areas for each individual.

(d) Bayesian analyses-- We used a maximum likelihood start for the Markov chain and an exponential hyperprior with a uniform distribution of 0 to 100. The chain was run for 41 million iterations, with a one million iteration burnin, and was sampled every 20,000 iterations. Each analysis was repeated three times to check for stability of the harmonic mean of the likelihoods. First, to determine the sequence and rates of transitions between pre-breeding, post-breeding, and dual molts we used the program MULTISTATE. Second, to determine the extent to which migratory tendencies influence the timing of molt, we tested for the presence and strength of correlated evolution and differences in evolutionary lability of molt and migration. Third, to test the hypothesis that the biochemical stability of the carotenoids in plumage varies with the timing of molt, we compared models of independent and dependent evolution. Our hypothesis would be supported if evolutionary transitions between stable and unstable plumage displays were correlated with molt timing. The second and third analyses were conducted using the program DISCRETE. Changes in stability of expressed carotenoids can be achieved through both metabolic modification and by selective expression of carotenoids in plumage (i.e., not depositing fast-degrading compounds or increasing the number of slow-degrading compounds deposited in the feathers). We examined correlation between these strategies and stability of expressed carotenoids using the program DISCRETE.

Appendix 1. Chemical stability of carotenoids in this study. Structural references are from Britton et al. [1] unless noted otherwise.

Carotenoid	Degradation group	Structural features⁹
(3R,3'R)-zeaxanthin	Fast	D, E
(3S,4R,3'R,6'R) 4-hydroxylutein	Fast	B, D, E
(3S,4R,3'S,6'R) 4-hydroxylutein	Fast	B, D, E
3'-dihydrolutein	Fast	B, D, E, H
7,8-dihydro β -cryptoxanthin ²	Fast	D, E
7,8-dihydro-lutein	Fast	B, D, E
7,8-dihydro-zeaxanthin ³	Fast	D, E
7,8,7',8'-tetrahydro-zeaxanthin ²	Fast	D, E
9-Z-7,8-dihydro-lutein ²	Fast	B, D, E, F
Anhydrolutein ⁴	Fast	B, D, E
Canary xanthophyll A ⁵	Fast	B, D, E, H
Canary xanthophyll B ⁵	Fast	B, D, H
Cis-lutein	Fast	B, D, E
Fucoxanthin	Fast	B, C, D, E
Lutein	Fast	B, D, E
Phoenicopterone	Fast	B, D, I
Piprioxanthin ⁶	Fast	B, D, E, H
α -carotene ¹	Fast	A, B
α -cryptoxanthin	Fast	B, D, E
α -isocryptoxanthin	Fast	B, D, E
β -carotene ¹	Fast	A
β -cryptoxanthin	Fast	D, E
β -isocryptoxanthin	Fast	D, E
(3S,3'S)-astaxanthin	Slow	D, I, J
3'-hydroxy-echinenone	Slow	D, E, I,
4-hydroxy-echinenone	Slow	D, E, I
4-oxo-gazianaxanthin ⁷	Slow	D, I, J, L
4-oxo-rubixanthin ⁷	Slow	D, I, J
Adonirubin	Slow	D, I, J
Adonixanthin	Slow	D, E, I, J
Canthaxanthin ⁸	Slow	D, I
Echinenone ⁸	Slow	D, I
Gazaniaxanthin	Slow	D, E, K
Papilioerythrinone	Slow	D, E, F
Rhodoxanthin ⁸	Slow	D, H, M
Rubixanthin	Slow	D, E, K
α -doradexanthin	Slow	B, D, E, I, J

Notes: 1: Experimental trials showed that these carotenoids degrade faster than carotenoids with substituted 6-carbon rings [2-5]. **2:** Stradi et al. 1998 [6]. **3:** Takaichi et al. 1996 [7]. **4:** McGraw et al. 2002 [8]. **5:** McGraw et al. 2001[9]. **6:** Hudon et al. 2007

[10]. **7:** Stradi et al. 1997 [11]. **8:** Mohamed et al. 2006 [12]. **9: Structural features and main references to stability categorization:** **A**–Unsubstituted 6-carbon rings. Absence of substituents on the end rings results in multiple potential sites for attack by reactive species. Cyclic end-groups are more reactive than the linear polyene chain, due to the fact that rings are twisted out of plane resulting in reduced co-planarity with the π -electron system of the polyene chain [13, 14]. **B**–Missing conjugated double bond in one (α -ionone) or both end-rings (ϵ -ionone). This increases reactivity by decreasing the number of conjugated double bonds [15]. **C**–Cumulative (consecutive) double bonds. Increases reactivity: adjacent double bonds are particularly reactive to light, air and oxygen, and are easily rearranged into a conjugated structure [16, 17]. **D**–Substituted 6-carbon rings. Provide a stabilizing effect when compared with unsubstituted 6-carbon rings, but strength of stabilization depends on the type of substituent and the position on the ring [15, 18]. **E**–Hydroxy-carotenoids: isolated –OH group in the end-ring. Hydroxylated carotenes are susceptible to oxidation and light degradation [19]. Reactivity is greater when substituents are in position C3 or C3' due to exposed hydrogens in position C4 and C4' that are allylic to the chromophore and highly reactive to oxidation [20, 21]. **F**–“Z” configuration of the polyene chain. Increases reactivity: The “Z” configuration is less stable than the “E” configuration because of steric strain, and making the molecule more vulnerable to degradation by oxygen and light [2, 22, 23]. **G**–Epoxy group: Increases reactivity due to ring strain. Fucoxanthin has several weakening groups contributing to its overall instability [16, 17]. **H**–Ketone group in ring position 3 or 3'. The double bond does not extend the conjugated π bonds system, and the reactive C4 and C-4' positions are exposed. The 3-keto carotenoids are easily reduced to unstable zeaxanthin [24]. **I**–Ketone group in ring position 4 and 4'. Decreases reactivity: Carbonyl groups increase the conjugation of the double bond system. They also allow the carotenoid to better quench free oxygen, stopping oxygen from reacting with and breaking the backbone, such that increasing numbers of carbonyl groups are associated with greater stability of carotenoids [21, 25, 26]. **J**–Alpha-hydroxy ketone (cyclic). Decreases reactivity: when exposed to heat the double bond of an alpha-hydroxy ketone is able to rearrange and produce an isomeric product. This means that instead of breaking under higher temperatures the ring undergoes rearrangement of electrons to maintain its overall structure [27]. **K**–Open ψ -end. Decreases reactivity [28]; **L**–“Z” configuration near the end does not affect the overall stability of the backbone. The stabilizing effect of the alpha-hydroxy ketone is greater and makes this carotenoid slow-degrading [29]. **M**–Retro-carotenoid. There is a shift in the position of the single and double bonds in the polyene chain [30, 31].

REFERENCES – APPENDIX 1

- [1] Britton, G., Liaaen-Jensen, S. & Pfander, H. 2004 *Carotenoids Handbook*. Birlin, Birkhauser.
- [2] Aparicio-Ruiz, R., Mínguez-Mosquera, M.I. & Gandul-Rojas, B. 2011 Thermal degradation kinetics of lutein, β -carotene and β -cryptoxanthin in virgin olive oils. *Journal of Food Composition and Analysis* **24**, 811-820. (doi:http://dx.doi.org/10.1016/j.jfca.2011.04.009).
- [3] Henry, L.K., Catignani, G.L. & Schwartz, S.J. 1998 Oxidative degradation kinetics of lycopene, lutein, and 9-cis and all-trans β -carotene. *J. Am. Oil Chem. Soc.* **75**, 823-829. (doi:10.1007/s11746-998-0232-3).
- [4] Ramel, F., Birtic, S., Cuiné, S., Triantaphylidès, C., Ravanat, J.-L. & Havaux, M. 2012 Chemical quenching of singlet oxygen by carotenoids in plants. *Plant Physiol.* (doi:10.1104/pp.111.182394).
- [5] Conn, P.F., Schalch, W. & Truscott, T.G. 1991 The singlet oxygen and carotenoid interaction. *J. Photochem. Photobiol. B: Biol.* **11**, 41-47. (doi:10.1016/1011-1344(91)80266-K).
- [6] Stradi, R., Hudon, J., Celentano, G. & Pini, E. 1998 Carotenoids in bird plumage: the complement of yellow and red pigments in true woodpeckers (Picinae). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **120**, 223-230. (doi: 10.1016/S0305-0491(98)10033-0).
- [7] Takaichi, S., Sandmann, G., Schnurr, G., Satomi, Y., Suzuki, A. & Misawa, N. 1996 The Carotenoid 7, 8-Dihydro- ψ end Group can be Cyclized by the Lycopene Cyclases from the Bacterium *Erwinia Uredovora* and the Higher Plant *Capsicum Annuum*. *Eur. J. Biochem.* **241**, 291-296. (doi:10.1111/j.1432-1033.1996.0291t.x).
- [8] McGraw, K.J., Adkins-Regan, E. & Parker, R.S. 2002 Anhydrolutein in the zebra finch: a new, metabolically derived carotenoid in birds. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **132**, 811-818. (doi: 10.1016/S1096-4959(02)00100-8).
- [9] McGraw, K.J., Hill, G.E., Stradi, R. & Parker, R.S. 2001 The influence of carotenoid acquisition and utilization on the maintenance of species-typical plumage pigmentation in male American goldfinches (*Carduelis tristis*) and northern cardinals (*Cardinalis cardinalis*). *Physiol. Biochem. Zool.* **74**, 843-852. (doi:doi:10.1086/323797).
- [10] Hudon, J., Anciães, M., Bertacche, V. & Stradi, R. 2007 Plumage carotenoids of the pin-tailed manakin (*Ilicura militaris*): evidence for the endogenous production of rhodoxanthin from a colour variant. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **147**, 402-411. (doi: 10.1016/j.cbpb.2007.02.004).
- [11] Stradi, R., Celentano, G., Boles, M. & Mercato, F. 1997 Carotenoids in Bird Plumage: The Pattern in a Series of Red-Pigmented Carduelinae. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **117**, 85-91. (doi:10.1016/S0305-0491(96)00271-4).
- [12] Mohamad, S.B., Yousef, Y.A., Melø, T.-B., Jávorfí, T., Partali, V., Sliwka, H.-R. & Naqvi, K.R. 2006 Singlet oxygen quenching by thione analogues of canthaxanthin, echinenone and rhodoxanthin. *J. Photochem. Photobiol. B: Biol.* **84**, 135-140.
- [13] Handelman, G.J. 1996 Carotenoids as scavengers of active oxygen species. In *Handbook of antioxidants* (eds. E. Cadenas & L. Packer), pp. 259-314. New York, Marcel Dekker, Inc.
- [14] El-Tinay, A. & Chichester, C. 1970 Oxidation of β -carotene. Site of initial attack. *The Journal of organic chemistry* **35**, 2290-2293.
- [15] Britton, G. 1995 Structure and properties of carotenoids in relation to function. *The FASEB Journal* **9**, 1551-1558.
- [16] Zhao, D., Kim, S.-M., Pan, C.-H. & Chung, D. 2014 Effects of heating, aerial exposure and illumination on stability of fucoxanthin in canola oil. *Food Chem.* **145**, 505-513. (doi:http://dx.doi.org/10.1016/j.foodchem.2013.08.045).
- [17] Piovan, A., Sergalia, R., Bresin, B., Rosy, C. & Filippini, R. 2013 Fucoxanthin from *Undaria pinnatifida*: photostability and coextractive effects. *Molecules* **18**, 6298-6310.
- [18] Yahia, E.M. & Ornelas-Paz, J.d.J. 2010 Chemistry, stability and biological actions of carotenoids. *Fruit and vegetable phytochemicals*, 177-222.

- [19] Arita, S., Otsuki, K., Osaki, K.-i., Murata, Y., Shimoishi, Y. & Tada, M. 2014 Reduction in photostability by the esterification of B-Cryptoxanthin. *Biosci., Biotechnol., Biochem.* **68**, 451-453.
- [20] Woodall, A.A., Lee, S.W.-M., Weesie, R.J., Jackson, M.J. & Britton, G. 1997 Oxidation of carotenoids by free radicals: relationship between structure and reactivity. *Biochimica et Biophysica Acta (BBA)-General Subjects* **1336**, 33-42.
- [21] Terao, J. 1989 Antioxidant activity of β -carotene-related carotenoids in solution. *Lipids* **24**, 659-661.
- [22] Mordi, R.C. 1993 Mechanism of beta-carotene degradation. *Biochem. J.* **292**, 310-312.
- [23] Etoh, H., Suhara, M., Tokuyama, S., Kato, H., Nakahigashi, R., Maejima, Y., Ishikura, M., Terada, Y. & Maoka, T. 2012 Auto-oxidation products of astaxanthin. *Journal of Oleo Science* **61**, 17-21.
- [24] Tausz, M., González-Rodríguez, Á.M., Wonisch, A., Peters, J., Grill, D., Morales, D. & Jiménez, M.S. 2004 Photostress, photoprotection, and water soluble antioxidants in the canopies of five Canarian laurel forest tree species during a diurnal course in the field. *Flora - Morphology, Distribution, Functional Ecology of Plants* **199**, 110-119. (doi:10.1078/0367-2530-00140).
- [25] Chabera, P., Fuciman, M., Hribek, P. & Polivka, T. 2009 Effect of carotenoid structure on excited-state dynamics of carbonyl carotenoids. *Physical Chemistry Chemical Physics* **11**, 8795-8803.
- [26] Maoka, T., Yasui, H., Ohmori, A., Tokuda, H., Suzuki, N., Osawa, A., Shindo, K. & Ishibashi, T. 2013 Anti-oxidative, anti-tumore-promoting, and anti-carcinogenic activities of adonirubin and adonixanthin. *Journal of Oleo Science* **62**, 181-186.
- [27] Rossbach, J., Harms, K. & Koert, U. 2015 alpha-Crotyl-alpha-difluoroboranyloxy-amides: Structure and Reactivity of Isolable Intermediates in Stereospecific α -Ketol Rearrangements. *Org. Lett.* **17**, 3122-3125. (doi:10.1021/acs.orglett.5b01427).
- [28] Sólyom, K., Maier, C., Weiss, J., Cocero, M.J., Mato, R.B., Carle, R. & Schweiggert, R. 2014 Structure–response relationship of carotenoid bioaccessibility and antioxidant activity as affected by the hydroxylation and cyclization of their terminal end groups. *Food Res. Int.* **66**, 107-114. (doi:http://dx.doi.org/10.1016/j.foodres.2014.09.004).
- [29] McMurry, J. 2004 *Organic Chemistry*. 6 ed. Belmont, CA, Thomas Learning.
- [30] Berg, C.J., LaFountain, A.M., Prum, R.O., Frank, H.A. & Tauber, M.J. 2013 Vibrational and electronic spectroscopy of the retro-carotenoid rhodoxanthin in avian plumage, solid-state films, and solution. *Archives of biochemistry and biophysics* **539**, 142-155.
- [31] Britton, G. 1993 Biosynthesis of carotenoids. In *Carotenoids in Photosynthesis* (eds. A.J. Young & G. Britton), pp. 96-126. Dordrecht, Springer Netherlands.

Appendix 2. Sources for data on molt timing, migration, and carotenoids expressed in plumage for the study species.

Species name	Common name	Molt	Migration	Carotenoids
<i>Aegithalos caudatus</i>	Long Tailed Tit	[1]	[2]	[3, 4]
<i>Agelaius phoeniceus</i>	Red-winged Blackbird	[5]	[6, 7]	[8-10]
<i>Amandava amandava</i>	Red Munia (Red Avadavat)	[11]	[11, 12]	[13]
<i>Amandava subflava</i>	Zebra Waxbill	[14]	[14]	[13]
<i>Bombycilla cedrorum</i>	Cedar Waxwing	[15]	[15, 16]	[3, 8, 17, 18]
<i>Bombycilla garrulus</i>	Bohemian Waxwing	[19, 20]	[16, 19, 20]	[3, 4]
<i>Bombycilla japonica</i>	Japanese Waxwing	[16]	[16]	[3]
<i>Bucanetes githagineus</i>	Trumpeter Finch	[11]	[11]	[4]
<i>Campephilus leucopogon</i>	Cream-backed Woodpecker	[21]	[21]	[22]
<i>Cardinalis cardinalis</i>	Northern Cardinal	[23]	[23]	[8, 24-26]
<i>Carduelis cannabina</i>	Linnet	[27]	[11]	[3, 28, 29]
<i>Carduelis carduelis</i>	European Goldfinch	[30-32]	[11]	[3, 33, 34]
<i>Carduelis chloris</i>	European Greenfinch	[31]	[11]	[33-36]
<i>Carduelis citrinella</i>	Citril Finch	[32]	[11]	[3, 34]
<i>Carduelis cucullata</i>	Red Siskin	[37]	[37]	[3, 4]
<i>Carduelis flammea</i>	Common Redpoll	[38]	[11]	[3, 39]
<i>Carduelis hornemanni</i>	Hoary Redpoll	[40]	[11]	[39]
<i>Carduelis sinica</i>	Oriental Greenfinch	[41]	[41]	[34]
<i>Carduelis spinoides</i>	Yellow-Breasted Greenfinch	[42]	[42]	[34]
<i>Carduelis spinus</i>	Eurasian Siskin	[11]	[11]	[3, 33, 34]
<i>Carduelis tristis</i>	American Goldfinch	[43]	[43]	[8, 44]
<i>Carpodacus mexicanus</i>	House Finch	[45]	[45]	[29, 46-50]
<i>Carpodacus pulcherrimus</i>	Beautiful Rosefinch	[51]	[51]	[4, 39]
<i>Carpodacus roseus</i>	Pallas' Rosefinch	[11]	[11]	[3, 28]
<i>Carpodacus trifasciatus</i>	Three-banded Rosefinch	[51]	[51]	[4, 39]
<i>Chlorospingus pileatus</i>	Sooty-capped Bush Tanager	[52]	[47, 53]	[52]
<i>Coccothraustes vespertinus</i>	Evening Grosbeak	[54]	[11, 54]	[55]
<i>Coereba flaveola</i>	Bananaquit	[56]	[11]	[56]
<i>Colaptes auratus</i>	Northern Flicker	[57]	[57, 58]	[3]
<i>Colaptes campestris</i>	Campo Flicker	[21]	[21]	[22]
<i>Colaptes chrysoides</i>	Gilded Flicker	[59]	[59]	[3]
<i>Dendrocopos major</i>	Great Spotted Woodpecker	[6]	[6]	[3, 22]
<i>Dendroica coronata</i>	Yellow-rumped Warbler	[60, 61]	[11, 60]	[10]
<i>Dendroica palmarum</i>	Palm Warbler	[62]	[62]	[10]
<i>Dendroica petechia</i>	Yellow Warbler	[61, 63]	[63]	[55]
<i>Dryocopus pileatus</i>	Pileated Woodpecker	[64]	[64]	[22]
<i>Emberiza citrinella</i>	Yellowhammer	[65]	[65]	[3, 4]
<i>Emberiza melanocephala</i>	Black-headed Bunting	[65]	[65]	[3, 4]

<i>Erithacus rubecula</i>	European Robin	[16]	[16]	[4]
<i>Erythrura gouldiae</i>	Gouldian Finch	[66]	[66]	[4]
<i>Erythrura psittacea</i>	Red-headed Parrot Finch	[67]	[67]	[4]
<i>Eudocimus ruber</i>	Scarlet Ibis	[68]	[69]	[70]
<i>Euplectes afer</i>	Yellow-crowned Bishop	[71]	[71, 72]	[4, 73]
<i>Euplectes ardens</i>	Red-collared Widowbird	[74, 75]	[76]	[77]
<i>Euplectes axillaris</i>	Red-shouldered Widowbird	[74, 75]	[73]	[75]
<i>Euplectes capensis</i>	Yellow Bishop	[78, 79]	[79, 80]	[4]
<i>Euplectes macroura</i>	Yellow-mantled Widowbird	[77, 81]	[81, 82]	[77]
<i>Euplectes orix</i>	Southern Red Bishop	[83]	[83]	[4, 73]
<i>Ficedula zanthopygia</i>	Korean Flycatcher	[84, 85]	[86]	[4]
<i>Foudia madagascariensis</i>	Red Fody	[87]	[88, 89]	[4, 88]
<i>Fringilla coelebs</i>	Chaffinch	[31, 75]	[11]	[3]
<i>Fringilla montifringilla</i>	Brambling	[90]	[11]	[10]
<i>Geothlypis trichas</i>	Common Yellowthroat	[61, 91]	[91]	[55, 92]
<i>Icteria virens</i>	Yellow-breasted Chat	[93]	[93]	[94]
<i>Icterus galbula</i>	Northern (Baltimore) Oriole	[95]	[95]	[24]
<i>Ilicura militaris</i>	Pin-tailed Manakin	[96]	[97]	[98]
<i>Larus delawarensis</i>	Ring-billed Gull	[99]	[99]	[100]
<i>Larus pipixcan</i>	Franklin's Gull	[101]	[101]	[100]
<i>Leiothrix argentauris</i>	Silver-eared Mesia	[102]	[103]	[4]
<i>Leiothrix lutea</i>	Pekin Robin	[104]	[105]	[3, 4]
<i>Loxia curvirostra</i>	Red Crossbill	[106]	[11, 106]	[3, 28, 33, 107, 108]
<i>Loxia leucoptera</i>	White-winged Crossbill	[109]	[7, 109]	[3, 24, 107, 110]
<i>Luscinia calliope</i>	Siberian Rubythroat	[111]	[16]	[3, 4]
<i>Malurus melanocephalus</i>	Red-backed Fairy- Wren	[112-114]	[115, 116]	[117]
<i>Masius chrysopterus</i>	Golden-winged Manakin	[96, 118]	[97]	[98]
<i>Melanerpes candidus</i>	White Woodpecker	[21]	[21]	[22]
<i>Melanerpes lewis</i>	Lewis's Woodpecker	[119]	[119, 120]	[22]
<i>Motacilla flava</i>	Yellow Wagtail	[121]	[122]	[4]
<i>Mycerobas carnipes</i>	White-winged Grosbeak	[123, 124]	[123]	[10]
<i>Mycerobas icteroides</i>	Black-and-yellow Grosbeak	[125]	[126]	[10]
<i>Neochmia ruficauda</i>	Star Finch	[127]	[127]	[13]
<i>Nesospiza acunhae</i>	Tristan Bunting	[128]	[65]	[128]
<i>Notiomystis cincta</i>	Hihi (Stitchbird)	[129]	[129]	[130, 131]
<i>Oriolus oriolus</i>	Golden Oriole	[75]	[75]	[4]
<i>Oriolus xanthornus</i>	Black-Hooded Oriole	[65]	[65]	[4]
<i>Parus ater</i>	Coal Tit	[2]	[2]	[3]
<i>Parus caeruleus</i>	Blue Tit	[132]	[132]	[3, 133]

<i>Parus major</i>	Great Tit	[31, 132]	[134]	[3, 135-140]
<i>Parus spilonotus</i>	Yellow-cheeked Tit	[141]	[142]	[4]
<i>Pericrocotus flammeus</i>	Scarlet Minivet	[11]	[11]	[3]
<i>Pheucticus ludovicianus</i>	Rose-breasted Grosbeak	[11, 143]	[11, 143]	[8, 24]
<i>Phoeniconaias minor</i>	Lesser Flamingo	[144]	[144]	[145]
<i>Phoenicoparrus andinus</i>	Andean Flamingo	[146]	[141, 147]	[148]
<i>Phoenicoparrus jamesi</i>	James's Flamingo	[146]	[147]	[148]
<i>Phoenicopterus chilensis</i>	Chilean Flamingo	[146]	[141, 147]	[145]
<i>Phoenicopterus roseus</i>	Greater Flamingo	[146, 149, 150]	[11, 151]	[148]
<i>Phoenicopterus ruber</i>	American Flamingo	[146]	[146]	[148, 152]
<i>Picooides tridactylus</i>	Three-toed Woodpecker	[6]	[11, 153]	[22]
<i>Picooides villosus</i>	Hairy Woodpecker	[6]	[6]	[22]
<i>Picus viridis</i>	Green Woodpecker	[6]	[6]	[22]
<i>Pinicola enucleator</i>	Pine Grosbeak	[154]	[11, 154]	[3, 4, 28, 33, 107]
<i>Pipra chloromeros</i>	Round-tailed Manakin	[96]	[97]	[155]
<i>Pipra erythrocephala</i>	Golden-headed Manakin	[156]	[97, 156]	[155]
<i>Pipra rubrocapilla</i>	Red-headed Manakin	[96, 157]	[97]	[155]
<i>Piranga flava</i>	Hepatic Tanager	[158]	[158]	[24]
<i>Piranga ludoviciana</i>	Western Tanager	[159]	[159]	[24]
<i>Piranga olivacea</i>	Scarlet Tanager	[160]	[160]	[8, 24]
<i>Piranga rubra</i>	Summer Tanager	[161]	[161]	[24]
<i>Platalea ajaja</i>	Roseate Spoonbill	[162]	[162]	[4, 163]
<i>Ploceus bicolor</i>	Forest Weaver	[164]	[164]	[4]
<i>Ploceus capensis</i>	Cape Weaver	[75, 78, 164]	[75]	[4]
<i>Ploceus cucullatus</i>	Village Weaver	[71]	[11]	[4, 165]
<i>Ploceus philippinus</i>	Baya Weaver	[166]	[166]	[4]
<i>Ploceus sakalava</i>	Sakalava Weaver	[167]	[168]	[4]
<i>Ploceus velatus</i>	African Masked Weaver	[83]	[83]	[4]
<i>Pyrrhula pyrrhula</i>	Eurasian Bullfinch	[11]	[11]	[3, 28]
<i>Quelea cardinalis</i>	Cardinal Quelea	[71, 72]	[71, 72]	[4]
<i>Quelea erythrops</i>	Red-headed Quelea	[75, 164]	[169]	[4]
<i>Quelea quelea</i>	Red-billed Quelea	[83]	[83]	[4]
<i>Regulus regulus</i>	Goldcrest	[170]	[170]	[3]
<i>Regulus satrapa</i>	Golden-crowned Kinglet	[171]	[171]	[172]
<i>Rhodopechys obsoletus</i>	Desert Finch	[11]	[11]	[3, 4]
<i>Serinus canaria</i>	Common Canary	[11]	[11]	[4, 165]
<i>Serinus mozambicus</i>	Yellow-fronted Canary	[71]	[71]	[3, 4]
<i>Serinus pusillus</i>	Red-fronted Serin	[11]	[11]	[3, 29, 34]
<i>Serinus serinus</i>	European Serin	[11]	[11]	[3, 34]
<i>Setophaga ruticilla</i>	American Redstart	[61, 173, 174]	[61, 173]	[10]
<i>Sicalis flaveola</i>	Saffron Finch	[175, 176]	[176]	[10]

<i>Sphyrapicus varius</i>	Yellow-bellied Sapsucker	[177]	[177]	[22]
<i>Sterna elegans</i>	Elegant Tern	[6]	[6]	[178]
<i>Telophorus sulfureopectus</i>	Sulfur-breasted Bushshrike	[75]	[179]	[4]
<i>Tichodroma muraria</i>	Wallcreeper	[121]	[180]	[3]
<i>Uragus sibiricus</i>	Long-tailed Rosefinch	[51]	[181]	[3, 4, 28, 39]
<i>Vermivora ruficapilla</i>	Nashville Warbler	[61, 182]	[182]	[183]
<i>Vermivora virginiae</i>	Virginia's Warbler	[184]	[184]	[183]
<i>Zosterops japonicus</i>	Japanese White-eye	[11, 185]	[186]	[3]

REFERENCES – APPENDIX 2

- [1] Hatchwell, B.J., Gullett, P.R. & Adams, M.J. 2014 Helping in cooperatively breeding long-tailed tits: a test of Hamilton's rule. *Philosophical Transactions of the Royal Society B* **369**, 20130565. (doi:10.1098/rstb.2013.0565).
- [2] Cramp, S. & Perrins, C.M. 1993 *Handbook of the birds of Europe, the Middle East and North Africa: the birds of the Western Palearctic. Volume 7: Flycatchers to Shrikes*. Oxford, Oxford University Press.
- [3] Stradi, R. 1998 *The Colour of Flight: Carotenoids in Bird Plumage*. Milan, Italy, Solei Grupos Editoriale Informatico.
- [4] Stradi, R., Brambilla, L., Canali, G. & Mannucci, E. 1999 Colori in volo: il piumaggio degli uccelli. *Università degli Studi di Milano*, 117-146.
- [5] Yasukawa, K. & Searcy, W.A. 1995 Red-winged Blackbird (*Agelaius phoeniceus*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [6] Cramp, S. & Simmons, K.E.L. 1985 *Handbook of the birds of Europe, the Middle East and North Africa: the birds of the Western Palearctic. Volume 4, Terns to Woodpeckers*. Oxford, Oxford University Press.
- [7] Rohwer, S., Butler, L.K. & Froehlich, D.R. 2005 Ecology and Demography of East-West Differences in Molt Scheduling of Neotropical Migrant Passerines. In *Birds of two worlds: the ecology and evolution of migration* (eds. R. Greenberg & P.P. Marra), pp. 87-105, JHU Press.
- [8] Cohen, A., McGraw, K. & Robinson, W. 2009 Serum antioxidant levels in wild birds vary in relation to diet, season, life history strategy, and species. *Oecologia* **161**, 673-683.
- [9] McGraw, K.J., Wakamatsu, K., Clark, A.B. & Yasukawa, K. 2004 Red-winged blackbirds *Agelaius phoeniceus* use carotenoid and melanin pigments to color their epaulets. *J. Avian Biol.* **35**, 543-550.
- [10] McGraw, K.J. 2006 The mechanics of carotenoid coloration. In *Bird coloration. Volume 1: Mechanism and measurements* (eds. G.E. Hill & K.J. McGraw), pp. 177-242. Cambridge, MA, Harvard University Press.
- [11] Cramp, S. & Perrins, C.M. 1994 *Handbook of the birds of Europe, the Middle East, and North Africa: the birds of the Western Palearctic. Volume 8, Crows to Finches*. Oxford, Oxford University Press.
- [12] Langham, N.P.E. 1987 The annual cycle of the Avadavat *Amandava amandava* in Fiji. *Emu* **87**, 232-243.
- [13] McGraw, K.J. & Schuetz, J.G. 2004 The evolution of carotenoid coloration in estrildid finches: a biochemical analysis. *Comparative Biochemistry and Physiology Part B Biochemistry and Molecular Biology* **139**, 45-51.
- [14] King, T. 2011 The birds of the Lesio-Louna and Lefini Reserves, Batéké Plateau, Republic of Congo. *Malimbus* **33**, 1-41.
- [15] Witmer, M.C., Mountjoy, D.J. & Elliott, L. 2014 Cedar Waxwing (*Bombycilla cedrorum*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [16] Cramp, S. & Simmons, K.E.L. 1988 *Handbook of the birds of Europe, the Middle East and North Africa: the birds of the Western Palearctic. Volume 5, Tyrant Flycatchers to Thrushes*. Oxford, Oxford University Press.
- [17] Hudon, J. & Brush, A.H. 1989 Probable Dietary Basis of a Color Variant of the Cedar Waxwing. *J. Field Ornithol.* **60**, 361-368.
- [18] Brush, A.H. & Allen, K. 1963 Astaxanthin in the cedar waxwing. *Science* **142**, 47-48. (doi:10.1126/science.142.3588.47).
- [19] 2002 Bohemian waxwing. In *The International Wildlife Encyclopedia* (eds. M. Burton & R. Burton), p. 3168, 3rd ed, Marshall Cavendish.
- [20] Witmer, M.C. 2002 Bohemian Waxwing (*Bombycilla garrulus*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.

- [21] Short, L.L. & 1976 Notes on a collection of birds from the Paraguayan chaco. *American Museum Novitates* **2597**, 1-16.
- [22] Stradi, R., Hudon, J., Celentano, G. & Pini, E. 1998 Carotenoids in bird plumage: the complement of yellow and red pigments in true woodpeckers (Picinae). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **120**, 223-230. (doi: 10.1016/S0305-0491(98)10033-0).
- [23] Halkin, S.L. & Linville, S.U. 1999 Northern Cardinal (*Cardinalis cardinalis*). In *The Birds of North America Online* (ed. A. Poole).
- [24] Hudon, J. 1991 Unusual carotenoid use by the Western Tanager (*Piranga ludoviciana*) and its evolutionary implications. *Canadian Journal of Zoology* **69**, 2311-2320.
- [25] McGraw, K.J., Hill, G.E., Stradi, R. & Parker, R.S. 2001 The influence of carotenoid acquisition and utilization on the maintenance of species-typical plumage pigmentation in male American goldfinches (*Carduelis tristis*) and northern cardinals (*Cardinalis cardinalis*). *Physiol. Biochem. Zool.* **74**, 843-852. (doi:10.1086/323797).
- [26] McGraw, K.J., Hill, G.E. & Parker, R.S. 2003 Carotenoid pigments in a mutant cardinal: implications for the genetic and enzymatic control mechanisms of carotenoid metabolism in birds. *The Condor* **105**, 587-592.
- [27] Blanco, G., Frías, O., Garrido-Fernández, J. & Hornero-Méndez, D. 2005 Environmental-induced acquisition of nuptial plumage expression: a role of denaturation of feather carotenoproteins? *Proceedings of the Royal Society B* **272**, 1893-1900.
- [28] Stradi, R., Pini, E. & Celentano, G. 2001 Carotenoids in bird plumage: the complement of red pigments in the plumage of wild and captive bullfinch (*Pyrrhula pyrrhula*). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **128**, 529-535.
- [29] Badyaev, A.V., Belloni, V., Kennedy, L. & Delaney, R. Unpublished Data.
- [30] Crowe, O., Coombes, R.H., Lysaght, L., O'Brien, C., Choudhury, K.R., Walsh, A.J., Wilson, J.H. & O'Halloran, J. 2010 Population trends of widespread breeding birds in the Republic of Ireland 1998-2008. *Bird Study* **57**, 267-280. (doi:10.1080/00063651003615147).
- [31] De La Hera, I.D., Schaper, S.V., Díaz, J.A., Pérez-Tris, J., Bensch, S. & Tellería, J.L. 2011 How much variation in the molt duration of passerines can be explained by the growth rate of tail feathers? *The Auk* **128**, 321-329.
- [32] Borrás, A., Cabrera, T., Cabrera, J. & Senar, J.C. 2004 Interlocality variation in speed of moult in the citril finch *Serinus citrinella*. *Ibis* **146**, 14-17.
- [33] Stradi, R., Celentano, G. & Nava, D. 1995 Separation and identification of carotenoids in bird's plumage by high-performance liquid chromatography-diode-array detection. *Journal of Chromatography B: Biomedical Sciences and Applications* **670**, 337-348.
- [34] Stradi, R., Celentano, G., Rossi, E., Rovati, G. & Pastore, M. 1995 Carotenoids in bird plumage—I. The carotenoid pattern in a series of palearctic carduelinae. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **110**, 131-143.
- [35] Peters, A., Delhey, K., Andersson, S., Van Noordwijk, H. & Förchler, M.I. 2008 Condition-dependence of multiple carotenoid-based plumage traits: an experimental study. *Funct. Ecol.* **22**, 831-839.
- [36] Saks, L., McGraw, K. & Hórak, P. 2003 How feather colour reflects its carotenoid content. *Funct. Ecol.* **17**, 555-561.
- [37] Coats, S. & Phelps, W.H., Jr. 1985 The venezuelan red siskin: case history of an endangered species. *Ornithological Monographs* **36**, 977-985.
- [38] Knox, A.G. & Lowther, P.E. 2000 Common Redpoll (*Acanthis flammea*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [39] Stradi, R., Celentano, G., Boles, M. & Mercato, F. 1997 Carotenoids in Bird Plumage: The Pattern in a Series of Red-Pigmented Carduelinae. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **117**, 85-91. (doi:10.1016/S0305-0491(96)00271-4).
- [40] Knox, A.G. & Lowther, P.E. 2000 Hoary Redpoll (*Acanthis hornemanni*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.

- [41] Nakamura, H. 1979 Summer concentration and moult in the oriental greenfinch *Garcluelis sinica*. *Japanese Journal of Ornithology* **28**, 1-27. (doi:10.3838/jjo1915.28.1).
- [42] Senar, J.C., Negro, J.J., Quesada, J., Ruiz, I. & Garrido, J. 2008 Two pieces of information in a single trait? The yellow breast of the great tit (*Parus major*) reflects both pigment acquisition and body condition. *Behaviour* **145**, 1195-1210.
- [43] McGraw, K.J. & Middleton, A.L. 2009 American Goldfinch (*Spinus tristis*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [44] McGraw, K., Hill, G.E. & Parker, R.S. 2005 The physiological costs of being colourful: nutritional control of carotenoid utilization in the American goldfinch, *Carduelis tristis*. *Anim. Behav.* **69**, 653-660.
- [45] Badyaev, A.V., Belloni, V. & Hill, G.E. 2012 House Finch (*Haemorhous mexicanus*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [46] Inouye, C.Y., Hill, G.E., Stradi, R.D., Montgomerie, R. & Bosque, C. 2001 Carotenoid pigments in male house finch plumage in relation to age, subspecies, and ornamental coloration. *The Auk* **118**, 900-915.
- [47] Brush, A.H. & Power, D.M. 1976 House finch pigmentation: carotenoid metabolism and the effect of diet. *The Auk* **93**, 725-739.
- [48] Toomey, M.B. & McGraw, K.J. 2009 Seasonal, sexual, and quality related variation in retinal carotenoid accumulation in the house finch (*Carpodacus mexicanus*). *Funct. Ecol.* **23**, 321-329.
- [49] Toomey, M.B., Butler, M.W. & McGraw, K.J. 2010 Immune-system activation depletes retinal carotenoids in house finches (*Carpodacus mexicanus*). *J. Exp. Biol.* **213**, 1709-1716. (doi:10.1242/jeb.041004).
- [50] McGraw, K.J., Nolan, P.M. & Crino, O.L. 2006 Carotenoid accumulation strategies for becoming a colourful House Finch: analyses of plasma and liver pigments in wild moulting birds. *Funct. Ecol.* **20**, 678-688. (doi:10.1111/j.1365-2435.2006.01121.x).
- [51] Clement, P., Harris, A. & Davis, J. 1993 *Finches & Sparrows: an identification guide*, Princeton University Press.
- [52] Johnson, N.K. & Brush, A.H. 1972 Analysis of Polymorphism in the Sooty-Capped Bush Tanager. *Systematic Zoology* **21**, 245-262.
- [53] Hilty, S., Ln: del Hoyo, J., Elliott, A., Sargatal, J., Christie, D.A. & De Juana, E. 2011 Sooty-capped bush-tanager (*Chlorospingus pileatus*). *Handbook of the Birds of the World Alive*.
- [54] Gillihan, S.W. & Byers, B. 2001 Evening Grosbeak (*Coccothraustes vespertinus*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [55] McGraw, K.J., Beebee, M.D., Hill, G.E. & Parker, R.S. 2003 Lutein-based plumage coloration in songbirds is a consequence of selective pigment incorporation into feathers. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **135**, 689-696.
- [56] Hudon, J., Ouellet, H., Bénito-Espinal, É. & Brush, A.H. 1996 Characterization of an orange variant of the Bananaquit (*Coereba flaveola*) on La Désirade, Guadeloupe, French West Indies. *The Auk* **113**, 715-718.
- [57] Wiebe, K.L. & Moore, W.S. 2008 Northern Flicker (*Colaptes auratus*). In *The Birds of North America Online* (ed. A. Poole), Ithaca: Cornell Lab of Ornithology.
- [58] Wunderle, J.M., Jr. 1982 The timing of the breeding season in the Bananaquit (*Coereba flaveola*) on the Island of Grenada, W.I. *Biotropica* **14**, 124-131.
- [59] Moore, W.S. 1995 Gilded flicker (*Colaptes chrysoides*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [60] Hunt, P.D. & Flaspohler, D.J. 1998 Yellow-rumped Warbler (*Setophaga coronata*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [61] Carlisle, J.D., Kaltenecker, G.S., Swanson, D.L. & Brittingham, M. 2005 Molt strategies and age differences in migration timing among autumn landbird migrants in southwestern Idaho. *The Auk* **122**, 1070-1085.

- [62] Herbert, W.J.W. 2013 Palm Warbler (*Setophaga palmarum*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [63] Lowther, P.E., Celada, C., Klein, N.K., Rimmer, C.C. & A., S.D. 1999 Yellow Warbler (*Setophaga petechia*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [64] Bull, E.L. & Jackson, J.A. 2011 Pileated Woodpecker (*Dryocopus pileatus*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [65] Cramp, S., Perrins, C.M. & Brooks, D.J. 1994 *Handbook of the Birds of Europe, the Middle East and North Africa: the birds of the Western Palearctic. Volume 9, Buntings and New World Warblers*. Oxford, Oxford University Press.
- [66] O'Malley, C. 2006 National Recovery Plan for the Gouldian Finch (*Erythrura gouldiae*). (Palmerston, WWF-Australia, Sydney and Parks and Wildlife NT, Department of Natural Resources, Environment and the Arts, NT Government.
- [67] Clement, P., Harris, A. & Davis, J. 1999 *Finches and sparrows*. London, Christopher Helm.
- [68] Amadon, D. 1966 Avian plumages and molts. *The Condor* **68**, 263-278. (doi:10.2307/1365560).
- [69] Phelps, K. 2004 *Eudocimus ruber*, scarlet ibis. *Animal Diversity Web*.
- [70] Fox, D.L. 1962 Carotenoids of the scarlet ibis. *Comparative Biochemistry and Physiology* **5**, 31-43.
- [71] Benson, C.W. & Irwin, M.P.S. 1967 *A contribution to the ornithology of Zambia*, London, published on behalf of the National Museum of Zambia by Oxford U.P.
- [72] Craig, A.J.F.K. 2010 Family Ploceidae (Weavers). In *Handbook of the Birds of the World. Weavers to New World Warblers*. (J. del Hoyo, Elliott, A., Christie, D.A. (eds)), pp. 74-197. Barcelona, Lynx Edicions.
- [73] Prager, M., Johansson, E.I.A. & Andersson, S. 2009 Differential ability of carotenoid C4-oxygenation in yellow and red bishop species (*Euplectes* spp.). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **154**, 373-380.
- [74] Oschadleus, H.D. & Underhill, L.G. 2006 Breeding seasonality and primary moult parameters of *Euplectes* species in South Africa. *Ostrich* **77**, 142-152.
- [75] Craig, A.J.F.K. 1983 Moults in southern african passerine birds: a review. *Ostrich* **54**, 220-237.
- [76] Pryke, S.R., Andersson, S., Lawes, M.J. & Piper, S.E. 2002 Carotenoid status signaling in captive and wild red-collared widowbirds: independent effects of badge size and color. *Behav. Ecol.* **13**, 622-631.
- [77] Andersson, S., Prager, M. & Johansson, E.I.A. 2007 Carotenoid content and reflectance of yellow and red nuptial plumages in widowbirds (*Euplectes* spp.). *Funct. Ecol.* **21**, 272-281.
- [78] Bonnevie, B.T. & Oschadleus, H.D. 2010 Timing of primary wing moult in sexually dimorphic passerines from the Western Cape, South Africa. *Ostrich* **81**, 63-67.
- [79] Craig, A.J.F.K. 1980 Behaviour and evolution in the genus *Euplectes*. *Journal of Ornithology* **121**, 144-161.
- [80] Lloyd, P. 2004 Comparative breeding success of yellow Bishop *Euplectes capensis* in two adjoining habitats. *Ostrich* **75**, 327-328.
- [81] Savalli, U.M. 1993 The timing of breeding and moult of the Yellow-mantled Widowbird *Euplectes macrourus* in western Kenya. *Ostrich* **64**, 40-56.
- [82] King, T. 2008 The birds of the Lesio-Louna and Lefini Reserves, Congo. (p. 103. Brazzaville, Congo.
- [83] Craig, A., Hulley, P.E., Whittington-Jones, C.A. & Bonnevie, B.T. 2001 Flying times and flight feathers: patterns of moult in sympatric seedeaters. *Ostrich*, 66-70.
- [84] La Touche, J.D.D. 1906 XXXVI.—Field-Notes on the Birds of Chinking, Lower Yangtse Basin.—Part II. *Ibis* **48**, 617-641.

- [85] Round, P.D. 2010 An analysis of records of three passage migrants in Thailand: Tiger Shrike *Lanius tigrinus*, Yellow-rumped Flycatcher *Ficedula zanthopygia* and Mugimaki Flycatcher *F. mugimaki*. *Forktail* **26**, 24-30.
- [86] Clement, P. & de Juana, E. 2014 Yellow-rumped Flycatcher (*Ficedula zanthopygia*). In *Handbook of the Birds of the World Alive* (eds. J. del Hoyo, A. Elliott, J. Sargatal, D.A. Christie & E. de Juana). Barcelona, Lynx Edicions.
- [87] Sueur, F. 1996 Observations ornithologiques à Madagascar. *Alauda* **64**, 435-442.
- [88] Estep, L.K., Shawkey, M.D. & G.E., H. 2006 Carotenoid-based breast plumage colour, body condition and clutch size in red fodies (*Foudia madagascariensis*). *Ostrich* **77**, 164-169.
- [89] Koenig, P. 2005 Sexing Madagascar Red Fody *Foudia madagascariensis* in the non-breeding period. *Afring News* **34**, 14-16.
- [90] Arkhipov, V.Y. 2005 Breeding biology of brambling (*Fringilla montifringilla* L.) in Taiga of the middle Yenisei. *Byulleten' Moskovskogo Obshchestva Ispytatelei Prirody Otdel Biologicheskii* **110**, 54-58.
- [91] Guzy, M.J. & Ritchison, G. 1999 Common Yellowthroat (*Geothlypis trichas*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [92] Cohen, A.A., Mauck, R.A., Wheelwright, N.T., Huntington, C.E. & McGraw, K.J. 2009 Complexity in relationships between antioxidants and individual life-history parameters in a seabird and a songbird. *Oikos* **118**, 1854-1861.
- [93] Eckerle, K.P. & Thompson, C.F. 2001 Yellow-breasted Chat (*Icteria virens*). In *The Birds of North America Online* (ed. A. Poole), Ithaca: Cornell Lab of Ornithology.
- [94] Mays, H.L.J., McGraw, K.J., Ritchison, G., Cooper, S., Rush, V. & Parker, R.S. 2004 Sexual dichromatism in the yellow-breasted chat *Icteria virens*: spectrophotometric analysis and biochemical basis. *J. Avian Biol.* **35**, 125-134.
- [95] Rising, J.D. & Flood, N.J. 1998 Baltimore Oriole (*Icterus galbula*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [96] Ryder, T.B. & Wolfe, J.D. 2009 The current state of knowledge on molt and plumage sequences in selected neotropical bird families: a review. *Ornitologia Neotropical* **20**, 1-18.
- [97] Snow, D.W. 2004 Pipridae: family account. In *Handbook of the birds of the world: Cotingas to pipits and wag-tails*. Barcelona, Lynx Edicions.
- [98] Hudon, J., Anciães, M., Bertacche, V. & Stradi, R. 2007 Plumage carotenoids of the pin-tailed manakin (*Ilicura militaris*): evidence for the endogenous production of rhodoxanthin from a colour variant. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **147**, 402-411. (doi:http://dx.doi.org/10.1016/j.cbpb.2007.02.004).
- [99] Pollet, I.L., Shutler, D., Chardine, J. & Ryder, J.P. 2012 Ring-billed Gull (*Larus delawarensis*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [100] McGraw, K.J. & Hardy, L.S. 2006 Astaxanthin is responsible for the pink plumage flush in Franklin's and Ring-billed gulls. *J. Field Ornithol.* **77**, 29-33.
- [101] Burger, J. & Gochfeld, M. 2009 Franklin's Gull (*Leucophaeus pipixcan*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [102] Howard, R. & Moore, A. 1984 *A complete Checklist of the Birds of the World*. London, Macmillan.
- [103] Vince, M. 1996 *Softbills: Care, breeding and conservation*. Blaine, WA, Hancock House.
- [104] Male, T.D., Steven, G.F. & Ralph, C.J. 1998 Red-billed Leiothrix (*Leiothrix lutea*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [105] Ralph, C.J., Fancy, S.G. & Male, T.D. 1998 Demography of an introduced red-billed Leiothrix population in Hawaii. *The Condor* **100**, 468-473.
- [106] Adkisson, C.S. 1996 Red Crossbill (*Loxia curvirostra*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.

- [107] Stradi, R., Rossi, E., Celentano, G. & Bellardi, B. 1996 Carotenoids in bird plumage: The pattern in three *Loxia* species and in *Pinicola enucleator*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **113**, 427-432.
- [108] Del Val, E., Senar, J.C., Garrido-Fernandez, J., Jaren, M., Borrás, A., Cabrera, J. & Negro, J.J. 2009 The liver but not the skin is the site for conversion of a red carotenoid in a passerine bird. *Naturwissenschaften* **96**, 797-801.
- [109] Benkman, C.W. 2012 White-winged Crossbill (*Loxia leucoptera*). In *The Birds of North America* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [110] Deviche, P., McGraw, K.J. & Underwood, J. 2008 Season-, sex-, and age-specific accumulation of plasma carotenoid pigments in free-ranging white-winged crossbills *Loxia leucoptera*. *J. Avian Biol.* **39**, 283-292.
- [111] Rhim, S.-J., Hur, W.-H., Lee, C.-B., Park, Y.-S., Choi, S.-Y. & Lee, W.-S. 2002 Characteristics of vegetation structure in breeding area of Siberian rubythroat (*Luscinia calliope*) in Daecheongbong peak, Mt. Seoraksan national park, South Korea. *Journal of Forestry Research (Harbin)* **13**, 239-240. (doi:10.1007/bf02871706).
- [112] Karubian, J. 2002 Costs and benefits of variable breeding plumage in the red-backed fairy-wren. *Evolution* **56**, 1673-1682.
- [113] Karubian, J., Swaddle, J.P., Varian-Ramos, C.W. & Webster, M.S. 2009 The relative importance of male tail length and nuptial plumage on social dominance and mate choice in the red-backed fairy-wren *Malurus melanocephalus*: evidence for the multiple receiver hypothesis. *J. Avian Biol.* **40**, 559-568.
- [114] Lindsay, W.R., Webster, M.S., Varian, C.W. & Schwabl, H. 2009 Plumage colour acquisition and behaviour are associated with androgens in a phenotypically plastic tropical bird. *Anim. Behav.*, 1525-1532.
- [115] Rowley, I. & Russell, E. 1997 *Fairy-Wrens and Grasswrens: Maluridae. Bird Families of the World volume 4*. Oxford, Oxford University Press.
- [116] Schodde, R. 1982 *The fairy-wrens: a monograph of the Maluridae*. Melbourne, Lansdowne Editions.
- [117] Rowe, M. & McGraw, K.J. 2009 Carotenoids in the Seminal Fluid of Wild Birds: Interspecific Variation in Fairy-Wrens. *The Condor* **110**, 694-700.
- [118] Hilty, S.L. & Brown, B. 1986 *A guide of birds of Colombia*, Princeton University Press.
- [119] Vierling, K.T., Saab, V.A. & Tobalske, B.W. 2013 Lewis's Woodpecker (*Melanerpes lewis*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [120] Tobalske, B.W. 1997 Lewis's woodpecker (*Melanerpes lewis*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [121] Blasco-Zumeta, J. & Heinze, G.M. 2012 Yellow Wagtail. In *Atlas de identificación de las aves de Aragón* (Obra Social de Ibercaja).
- [122] Cramp, S. 1993 *Handbook of the birds of Europe, the Middle East and North Africa: the birds of the Western Palearctic. Volume 6: Warblers*. Oxford, Oxford University Press.
- [123] Iovchenko, N.P. 1990 Ecology and adaptive peculiarities in annual cycle of the white-winged grosbeak (*Micropus carnipes*). *Ornitologiya* **24**, 84-94.
- [124] Iovchenko, N.P. 2006 Overlap of breeding and molt in a food specialist, the white-winged grosbeak. *Journal of Ornithology* **147**, 187-187.
- [125] AVIS-IBIS. 2010 Black And Yellow Grosbeak (*Perissospiza icteroides*).
- [126] Stanford, J.K. & Mayr., E. 1941 XVIII. The Vernay-cutting expedition to northern Burma. Part IV. *Ibis* **83**, 353-378.
- [127] Payne, R., Del Hoyo, J., Elliott, A., Sargatal, J., Christie, D.A. & De Juana, E. 2010 Star finch (*Neochmia ruficauda*). *Handbook of the Birds of the World Alive*.
- [128] Ryan, P.G., Moloney, C.L. & Hudon, J. 1994 Color variation and hybridization among *Nesospiza* buntings on inaccessible island, Tristan da Cunha. *Auk* **111**, 314-327.

- [129] Low, M., Castro, I. & Berggren, A. 2005 Cloacal erection promotes vent apposition during forced copulation in the new Zealand stitchbird (hihi): implications for copulation efficiency in other species. *Behav. Ecol. Sociobiol.* **58**, 247-255.
- [130] Ewen, J.G., Thorogood, R., Karadas, F., Pappas, A.C. & Surai, P.F. 2006 Influences of carotenoid supplementation on the integrated antioxidant system of a free living endangered passerine, the hihi (*Notiomystis cincta*). *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology* **143**, 149-154.
- [131] Ewen, J.G., Surai, P., Stradi, R., Møller, A.P., Vittorio, B., Griffiths, R. & Armstrong, D.P. 2006 Carotenoids, colour and conservation in an endangered passerine, the hihi or stitchbird (*Notiomystis cincta*). *Anim. Conserv.* **9**, 229-235.
- [132] Flegg, J.J.M. & Cox, C.J. 1969 The moult of British Blue Tit and Great Tit populations. *Bird Study* **16**, 147-157.
- [133] Arnold, K.E., Ramsay, S.L., Henderson, L. & Larcombe, S.D. 2010 Seasonal variation in diet quality: antioxidants, invertebrates and blue tits *Cyanistes caeruleus*. *Biol. J. Linn. Soc.* **99**, 708-717.
- [134] Delhey, K., Burger, C., Fiedler, W. & Peters, A. 2010 Seasonal changes in colour: a comparison of structural, melanin- and carotenoid-based plumage colours. *PLoS ONE* **5**, e11582. (doi:doi:10.1371/journal.pone.0011582).
- [135] Eeva, T., Sillanpää, S. & Salminen, J.P. 2009 The effects of diet quality and quantity on plumage colour and growth of great tit *Parus major* nestlings: a food manipulation experiment along a pollution gradient. *J. Avian Biol.* **40**, 491-499.
- [136] Hōrak, P., Surai, P.F., Ots, I. & Møller, A.P. 2004 Fat soluble antioxidants in brood-rearing great tits *Parus major*: relations to health and appearance. *J. Avian Biol.* **35**, 63-70.
- [137] Isaksson, C., Ornborg, J., Prager, M. & Andersson, S. 2008 Sex and age differences in reflectance and biochemistry of carotenoid-based colour variation in the great tit *Parus major*. *Biol. J. Linn. Soc.* **95**, 758-765.
- [138] Isaksson, C., Sturve, J., Almroth, B.C. & Andersson, S. 2009 The impact of urban environment on oxidative damage (TBARS) and antioxidant systems in lungs and liver of great tits, *Parus major*. *Environ. Res.* **109**, 46-50.
- [139] Quesada, J. & Senar, J.C. 2006 Comparing plumage colour measurements obtained directly from live birds and from collected feathers: the case of the great tit *Parus major*. *J. Avian Biol.* **37**, 609-616.
- [140] Sillanpää, S., Salminen, J.-P. & Eeva, T. 2009 Breeding success and lutein availability in great tit (*Parus major*). *Acta Oecol.* **35**, 805-810.
- [141] del Hoyo, J., Elliott, A. & Christie, D.A. 2007 *Handbook of the birds of the world*, Lynx Edicions.
- [142] Perrins, C.M. 1993 *L'encyclopédie mondiale des oiseaux*, Bordas Editions
- [143] Wyatt, V.E. & Francis, C.M. 2002 Rose-breasted Grosbeak (*Pheucticus ludovicianus*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [144] Brown, L.H. 1971 The breeding behavior of the lesser flamingo *Phoeniconaias minor*. *Ibis* **113**, 147-172. (doi:10.1111/j.1474-919X.1971.tb05141.x).
- [145] Fox, D.L., Smith, V.E. & Wolfson, A.A. 1967 Carotenoid selectivity in blood and feathers of lesser (African), chilean and greater (European) flamingos. *Comparative Biochemistry and Physiology* **23**, 225-232.
- [146] Shannon, P.W. 2000 Plumages and molt patterns in captive Caribbean flamingos. *Waterbirds: The International Journal of Waterbird Biology* **23**, 160-172. (doi:10.2307/1522161).
- [147] Bucher, E.H., Chani, J.M. & Echevarria, A.L. 2000 Andean flamingos breeding at Laguna Brava, La Rioja, Argentina. *Waterbirds* **23**, 119-120. (doi:10.2307/1522156).
- [148] Fox, D.L. & Hopkins, T.S. 1966 Comparative metabolic fractionation of carotenoids in three flamingo species. *Comparative Biochemistry and Physiology* **17**, 841-856.
- [149] Middlemiss, E. 1961 Notes on the greater flamingo. *Bokmakierie* **13**, 9-14.

- [150] Studer-Thiersch, A. 2000 What 19 years of observation on captive Greater Flamingos suggests about adaptations to breeding under irregular conditions. *Waterbirds* **23**, 150-159. (doi:10.2307/1522160).
- [151] Hamza, F., Hammouda, A., Chokri, M.A., Bechet, A. & Selmi, S. 2014 Distribution and abundance of greater flamingos *Phoenicopterus roseus* wintering in the central part of the Gulf of Gabes, Tunisia. *Alauda* **82**, 135-142.
- [152] Fox, D.L., Wolfson, A.A. & McBeth, J.W. 1969 Metabolism of β -carotene in the American flamingo, *Phoenicopterus ruber*. *Comparative Biochemistry and Physiology* **29**, 1223-1229.
- [153] Pechacek, P. 2004 Spacing behavior of eurasian Three-Toed Woodpeckers (*Picoides tridactylus*) during the breeding season in Germany. *Auk* **121**, 58-67.
- [154] Adkisson, C.S. 1999 Pine Grosbeak (*Pinicola enucleator*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [155] Hudon, J., Capparella, A.P. & Brush, A.H. 1989 Plumage pigment differences in manakins of the *Pipra erythrocephala* superspecies. *The Auk* **106**, 34-41.
- [156] Snow, D.W., Del Hoyo, J., Elliott, A., Sargatal, J., Christie, D.A. & De Juana, E. 2004 Golden-headed manakin (*Pipra erythrocephala*). *Handbook of the Birds of the World Alive*.
- [157] 2010 Red-headed Manakin (*Ceratopipra rubrocapilla*). In *Neotropical Birds Online* (ed. T.S. Schulenberg). Ithaca, Cornell Lab of Ornithology.
- [158] Eddleman, W.R. 2002 Hepatic Tanager (*Piranga flava*). In *Birds of North America Online* (Ithaca, Cornell Lab of Ornithology).
- [159] Hudon, J. 1999 Western Tanager (*Piranga ludoviciana*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [160] Mowbray, T.B. 1999 Scarlet Tanager (*Piranga olivacea*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [161] Robinson, W.D. 2012 Summer Tanager (*Piranga rubra*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [162] Dumas, J.V. 2000 Roseate Spoonbill (*Platalea ajaja*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [163] Fox, D.L., Hopkins, T.S. & Zilversmit, D.B. 1965 Blood carotenoids of the roseate spoonbill. *Comparative Biochemistry and Physiology* **14**, 641-649.
- [164] Oschadleus, H.D. 2005 Patterns of primary moult in the weavers, Ploceidae. Cape Town, University of Cape Town.
- [165] Brockmann, H. & Völker, O. 1934 Der gelbe Federfarbstoff des Kanarienvogels (*Serinus canaria canaria*) und das Vorkommen von Carotinoiden bei Vögeln. *Hoppe-Seyler's Zeitschrift für physiologische Chemie* **224**, 193-215.
- [166] Mathew, D.N. 1977 Molt in the baya weaver *Ploceus philippinus*. *J. Bombay Nat. Hist. Soc.* **74**, 233-245.
- [167] Sinclair, I. & Olivier, L. 2003 *Birds of the Indian Ocean Islands*, Struik publishers.
- [168] René de Roland, L.A. 2010 Madagascar Buzzard (*Buteo brachypterus*) nest in association with the colonial-nesting Sakalava Weaver (*Ploceus sakalava*). *Malagasy Nature* **4**, 65-66.
- [169] Carnaby, T. 2009 *Beat about the bush birds*, Jacana Media.
- [170] Martens, J., Päckert, M., Del Hoyo, J., Elliott, A., Sargatal, J., Christie, D.A. & De Juana, E. 2006 Goldcrest (*Regulus regulus*). *Handbook of the Birds of the World Alive*.
- [171] Swanson, D.L., Ingold, J.L. & Galati, R. 2012 Golden-crowned Kinglet (*Regulus satrapa*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [172] Chui, C.K.S., McGraw, K.J. & Doucet, S.M. 2011 Carotenoid-based plumage coloration in golden-crowned kinglets *Regulus satrapa*: pigment characterization and relationships with migratory timing and condition. *J. Avian Biol.* **42**, 309-322.
- [173] Sherry, T.W. & Holmes, R.T. 1997 American Redstart (*Setophaga ruticilla*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [174] Rohwer, S., Klein, P.W.J. & Heard, S. 1983 Delayed Plumage Maturation and the Presumed Prealternate Molt in American Redstarts. *Wilson Bull.* **95**, 199-208.

- [175] Palmerio, A.G. 2011 Parental care does not vary with age-dependent plumage in male Saffron Finches *Sicalis flaveola*. *Ibis* **153**, 421-424.
- [176] Palmerio, A.G. & Massoni, V. 2009 Reproductive Biology of Female Saffron Finches does not Differ by the Plumage of the Mate. *Condor* **111**, 715-721.
- [177] Walters, E.L., Miller, E.H. & Lowther, P.E. 2002 Yellow-bellied Sapsucker (*Sphyrapicus varius*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [178] Hudon, J. & Brush, A.H. 1990 Carotenoids Produce Flush in the Elegant Tern Plumage. *The Condor* **92**, 798-801.
- [179] Hockey, P.A.R., Dean, W.R.J. & P.G., R. 2005 *Roberts Birds of southern Africa*. 7th ed. Cape Town, John Voelcker Bird Book Fund.
- [180] Saniga, M. 1995 Seasonal distribution, habitat, and territory of wallcreeper (*Tichodroma muraria*) in the Velkafatra mountains (West Carpathians). *Biologia* **50**, 195-202.
- [181] Zhaohui, X. & Shichun, L. 1994 Studies on the breeding ecology of the long-tailed rosefinch. *Acta Ecologica Sinica* **14**, 57-61.
- [182] Lowther, P.E. & Williams, J.M. 2011 Nashville Warbler (*Oreothlypis ruficapilla*) In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [183] Brush, A.H. & Johnson, N.K. 1976 The evolution of color differences between Nashville and Virginia's Warblers. *The Condor* **78**, 412-414.
- [184] Olson, C.R. & Martin, T.E. 1999 Virginia's warbler (*Oreothlypis virginiae*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [185] Guest-Van Riper, S. 2000 Japanese White-eye (*Zosterops japonicus*). In *The Birds of North America Online* (ed. A. Poole). Ithaca, Cornell Lab of Ornithology.
- [186] Brazil, M. 2009 *Birds of East Asia: eastern China, Taiwan, Korean, Japan, eastern Russia*. London, Christopher Helm.

Table S1. Analysis of covariance of independent linear contrasts of carotenoid and molt parameters.

Dependent variable	Covariates			
	Migratory status		Geographic distribution	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Slow degrading carotenoids	0.18	0.67	4.03	0.04*
Fast degrading carotenoids	2.68	0.08	3.71	<0.01*
Total carotenoids	2.04	0.15	3.32	0.01*
Stability, ratio	0.47	0.49	1.16	0.33
Selective expression, ratio	0.07	0.78	0.15	0.96
Metabolic elongation, reactions	5.85	0.01*	1.91	0.17
Time to molt, days	10.19	<0.01*	4.62	<0.01*