Stronger Historical Contingency Facilitates Ecological Specializations: An Example with Avian Carotenoid Networks

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ABSTRACT: Evolution requires both robustness of adaptive states and transitions between them. Understanding the mechanisms that reconcile these seemingly opposing properties is limited by the transient nature of evolutionary processes, where past pathways and contexts are often lost. Here, we overcome this limitation by tracing the biochemical evolution of avian carotenoid networks on the global carotenoid biochemical network, which is unmodified in avian evolution. By mapping enzymatic interactomes of 260 extant bird species and their reconstructed ancestral states onto this global network, we reveal that stepping stones between them are evolutionarily stable degenerate carotenoids—compounds that can be synthesized interchangeably by different dietary carotenoid–specific pathways. We find that ecological specialization across taxonomic groups is consistently associated with an uneven biochemical reach of individual dietary carotenoids, leading to increased fragmentation and reduced resilience of enzymatic networks to failure. However, the robustness of enzymatic networks of specialized groups is restored by the accumulation of degenerate carotenoids. This accumulation enables direct transitions between ecological specializations and sustains evolutionary explorations. Thus, the same feature of network structure—its degeneracy—increases the robustness of specialized enzymatic networks as enables evolutionary transitions between them. These findings provide an insight into the mechanistic basis for the interplay between natural selection and historical contingency, highlighting their fundamental interdependence.

Keywords: enzymatic networks, degeneracy, robustness, network resilience, network fragmentation, ecology, dietary specialization.

Introduction

Life's continuity requires the ability to transition between adaptations. The extent to which the strength, duration, or specificity of adaptations affect transitions between them is a central question in evolutionary theory (Fontana and Schuster 1998; Maynard Smith 1970; Stadler et al. 2001). The answer to this question has been traditionally framed as an interplay among necessity (optimization by natural selection), chance (random encounters of available pathways of change), and contingency (retention of past constraints and adaptations; Carroll 2001; Gould 2002; Stern and Orgogozo 2009). However, the fundamental interdependence of these processes limits our inference of the role of each one in evolution. For example, the contribution of chance depends on the accumulation of redundant pathways that can be taken at random at each evolutionary stage, whereas contingency is determined by the strength and duration of past functional associations (Shah et al. 2015; Xie et al. 2021; Park et al. 2022). Another difficulty comes from the singularity of the present: because evolution does not preserve either the paths it did not take or the historical contexts in which it occurred, the paths between past and present distributions are often interpreted as deterministic in retrospect, even when they were taken by transient chance events.

Overcoming these difficulties in understanding how life traverses adaptive states would inform us not only about the links between dominant evolutionary processes but also about the regulatory architecture that reconciles change with stability in development and evolution (Baldwin 1902; Schmalhausen 1949). In this goal, much effort has been devoted to re-creating and inferring the complete historical distribution and connectivity of past evolutionary states (Hansen 2006; de Visser and Krug 2014; Lässig et al. 2017; Starr et al. 2017). It is harder to infer past selection and contexts because these are always modified by evolution. Instead, insights into these missed processes are typically obtained by permutation of past and present—either by applying contemporary selection to a re-created distribution of evolutionary states and evaluating convergence or by inferring past processes from the size and distribution

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of current functional associations, for example, from complete knowledge of the fitness landscape (Lind et al. 2015; Sarkisyan et al. 2016; Xie et al. 2021; Gonzalez Somermeyer et al. 2022; Park et al. 2022). An alternative approach is to trace evolutionary change on a landscape of interconnected elements that are themselves not modified during evolution and where full knowledge of the connectivity of elements allows mapping of past and present evolutionary states. This approach has been widely used in the directed evolution research programs, where the pathways of desired steps or products are supplied externally to evolving entities in advance of their evolutionary change (e.g., Arnold 2019). Here, we use a conceptually similar approach to infer the patterns of avian subsampling of a global carotenoid biochemical network that originated largely in the context of early bacterial evolution.

Mapping all known carotenoid-converting biochemical networks of avian species onto the global carotenoid network of all known enzymatic reactions connecting naturally occurring carotenoids has revealed that birds utilize a small (<3%), insular, and interconnected space of the global network within which they repeatedly subsample and recombine biochemical modules (Badyaev et al. 2015; Morrison and Badyaev 2016*a*, 2018). Birds cannot synthesize carotenoids from noncarotenoids, so their traversing of the "avian part" of the global carotenoid network is anchored by the biochemical connectivity of dietary entry points—exogenous dietary carotenoids. Ecological groups of birds differ in these dietary entry points and their downstream enzymatic transformation (fig. 1*A*; Brush 1990; Britton 1998). This fundamental dependence on external inputs favors accumulation of enzymatic connectivity able to interchangeably convert an array of diverse dietary carotenoids into the same derived carotenoid—known as "degenerate carotenoids." Enzymatic degeneracy ensures the persistence of derived carotenoids by shielding them from environmental fluctuations and the associated evolutionary lability of any one of their exogenous precursors. In birds, this predictability underpins close evolutionary integration of externally derived carotenoids with feather proteins (Higginson et al. 2016; Potticary et al. 2020), ultimately producing an array of optical diversification of carotenoid-based plumage (Mendes-Pinto et al. 2012; LaFountain et al. 2015; Price-Waldman and Stoddard

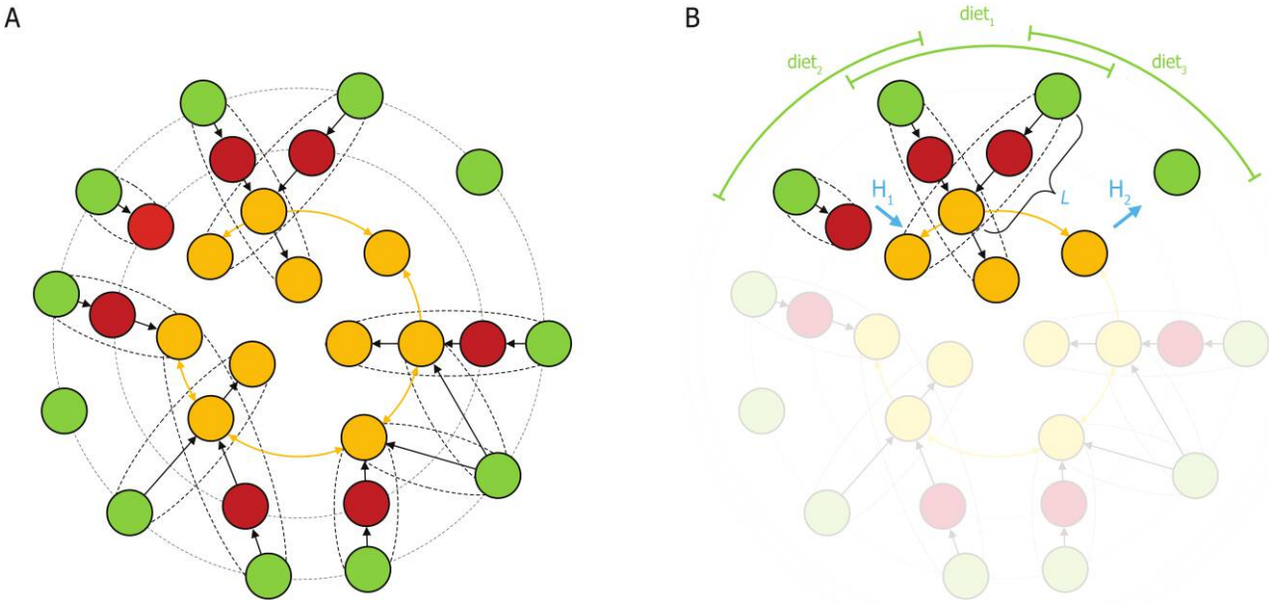

Figure 1: *A*, Avian carotenoid networks schematically depicted as three concentric groups of compounds: peripheral dietary carotenoids consumed unchanged with food (green circles; combination of these is dietary specialization), derived carotenoids (red circles; metabolically produced by enzymatic conversion [arrows] of dietary carotenoids), and degenerate carotenoids, located at the intersection of diet-specific biochemical modules or motifs (dashed ellipsoids) and shown in yellow. Figure S2 shows the actual avian carotenoid network according to this scheme. Degenerate carotenoids, produced interchangeably by distinct dietary carotenoids, are stepping stones in the evolution of avian carotenoid networks: they persist longer than dietary inputs, enable sustained exploration of downstream network connectivity, and directly link diet-specific and taxa-specific modules. *B*, Two general scenarios. Under the ecological revisitation scenario (H_1), the evolutionary persistence of degenerate compounds is a product of recurrent merging of diet-specific biochemical modules: the ecology-to-history path. Under the preadaptation scenario (H_2), degenerate compounds in ancestral networks enable attachment of novel dietary inputs and thus assure their retention: the history-to-ecology path. *L* is the distance (in reactions) between dietary and the most upstream degenerate carotenoids for each biochemical module merging into a degenerate compound.

2021). In evolution in general, degenerate carotenoids bridge diet-specific biochemical modules, form stepping stones in an evolutionary trajectory of lineages, and maintain cohesion of the global network while enabling subsampling and recombination of its parts (fig. 1*A*; Badyaev et al. 2015; Morrison and Badyaev 2016*a*). However, ecological groups of birds vary widely in their propensity to accumulate degenerate compounds in their networks because of differences in the enzymatic connectivity of their dietary carotenoids, the size and position of diet-specific biochemical motifs on the species' interactome, and the evolutionary persistence of dietary carotenoids (Badyaev et al. 2015). This variation enables us to examine network features associated with ecological specializations and evolutionary transitions between them. For example, a larger interconnected network encompassing an evolutionary state (e.g., a species or a dietary specialization) accumulates more potential pathways and thus may have a stronger effect on the subsequent evolutionary trajectory than a smaller, more modular network. Alternatively, more specialized modular networks could delineate most direct connectivity patterns and thus be more influential in long-term evolution (Wagner 2005, 2008; Johnson et al. 2019; Bakerlee et al. 2022; Gonzalez Somermeyer et al. 2022).

Although the low specificity of many carotenoid enzymes, likely arising from conservation of underlying genetic pathways (Mundy et al. 2016; Emerling 2018; Twyman et al. 2018; Price-Waldman and Stoddard 2021; Toomey et al. 2022), and the associated degeneracy of carotenoid networks is well known (Schmidt-Dannert et al. 2000; Umeno et al. 2005; Badyaev 2007; reviewed in Moise et al. 2022), how this degeneracy acquired such prominence in avian evolution (Badyaev et al. 2019) is poorly understood. Here, we combine a complete mapping of contemporary carotenoid networks of 260 species across 45 taxonomic families of birds with historical reconstruction of these networks over the last 20 million years to examine the association between the evolution of dietary specialization and the robustness of enzymatic networks underlying each dietary specialization. We envision two nonexclusive scenarios. Under the "ecological revisitation" scenario, degenerate compounds persist because of the recurrent historical merging of diet-specific biochemical modules (ecology-to-history path in fig. 1*B*). Alternatively, degenerate compounds can be gained and lost at the same rate as dietary- and nondegenerate-derived compounds, but their persistence in the ancestral network enables periodic attachment of additional dietary inputs; they thus form the "preadaptation" stage needed to restore network robustness in ongoing specialization and sustain its evolution (history-to-ecology path in fig. 1*B*).

We find that across manyfold differences in size and complexity among carotenoid-producing networks of ecological and taxonomic groups, the robustness of these networks are underpinned by the same principle: more ecologically specialized groups of birds have more fragmented metabolic networks that are less robust to failure and thus are expected to have shorter evolutionary persistence. Yet over time, the robustness of these networks is sustained by the accumulation of evolutionarily stable degenerate compounds produced by the recurrent merging of diet-specific biochemical motifs. Thus, the same aspect of network structure that enables the robustness of each functional state also facilitates evolutionary transitions between them. More generally, prior functional states prime subsequent ones, leading to constant updating of evolutionary trajectory to current ecological setting and illustrating how historical contingency facilitates fit to the current environment.

Material and Methods

Metabolic Network Building and Dietary Classification

Carotenoid metabolic networks were built for 260 species of birds based on an exhaustive list of all known carotenoid compounds found in the plumage, plasma, and bare parts and are given in table S1 and supplementary data S1 and S2 (tables S1–S4, supplementary data S1–S5, and supplementary code S1–S5 are available in the Harvard Dataverse at https://doi.org/10.7910/DVN/ES36IN; Morrison et al. 2025). We followed protocols of Badyaev et al. (2015) and Morrison and Badyaev (2016*a*) to map these species networks onto the "avian space" of the universal carotenoid metabolic network. These methods leverage the well-understood biochemical conversion of carotenoids in the global network and the known integument-deposited carotenoids in birds against incompletely studied intermediate enzymatic pathways in many avian species.

In brief, we first derived a global carotenoid metabolic network based on all biochemically documented enzymatic conversions of naturally occurring carotenoids in bacteria, plants, fungi, and animals (Badyaev et al. 2015). The avian space of this global carotenoid metabolic network was the subset of the reactions associated with the production of all carotenoid compounds biochemically identified in the diet, plumage, integument (bill, tarsi, skin), plasma, liver, fat, feces, and seminal fluid of birds (Badyaev et al. 2015; Morrison and Badyaev 2016*a*). Each of the 260 species networks was based on mapping the carotenoid compounds biochemically identified in the plumage, integument, or internal organs of males onto the avian space of the global carotenoid metabolic network (table S1; supplementary data S1, S2).

For each species, carotenoids were grouped into three categories based on their metabolic derivation prior to

feather deposition, following protocols in Badyaev et al. (2017): dietary carotenoids that are deposited into feathers unmodified, synthesized but nondegenerate carotenoids that can be derived only from a single dietary compound (hereafter, "derived carotenoids"), and degenerate carotenoids that can be interchangeably synthesized from more than one dietary precursor. The three groups of carotenoids and their corresponding enzymatic reactions are shown in figure S2 (figs. S1, S2 are available online).

Diet data (fig. 2) were collected from the primary literature and are given in table S1 and supplementary data S2. In brief, species diet was classified into five categories, following earlier studies of diet dependency in carotenoid pigmentation (e.g., Gray 1996; Matsuno 2001; Mahler et al. 2003; Olson and Owens 2005; McGraw 2006): plants and seeds (terrestrial and/or aquatic plants, seeds, nuts, woods, or grains), fruits and nectar (fruit, flowers, nectar, pollen, or sap), invertebrates (terrestrial and/or aquatic invertebrates, including insects, worms, and caterpillars), vertebrates (nonaquatic and/or aquatic vertebrates, including carrion, fish, and shrimp), and omnivore (combination of at least two of the other diet classifications).

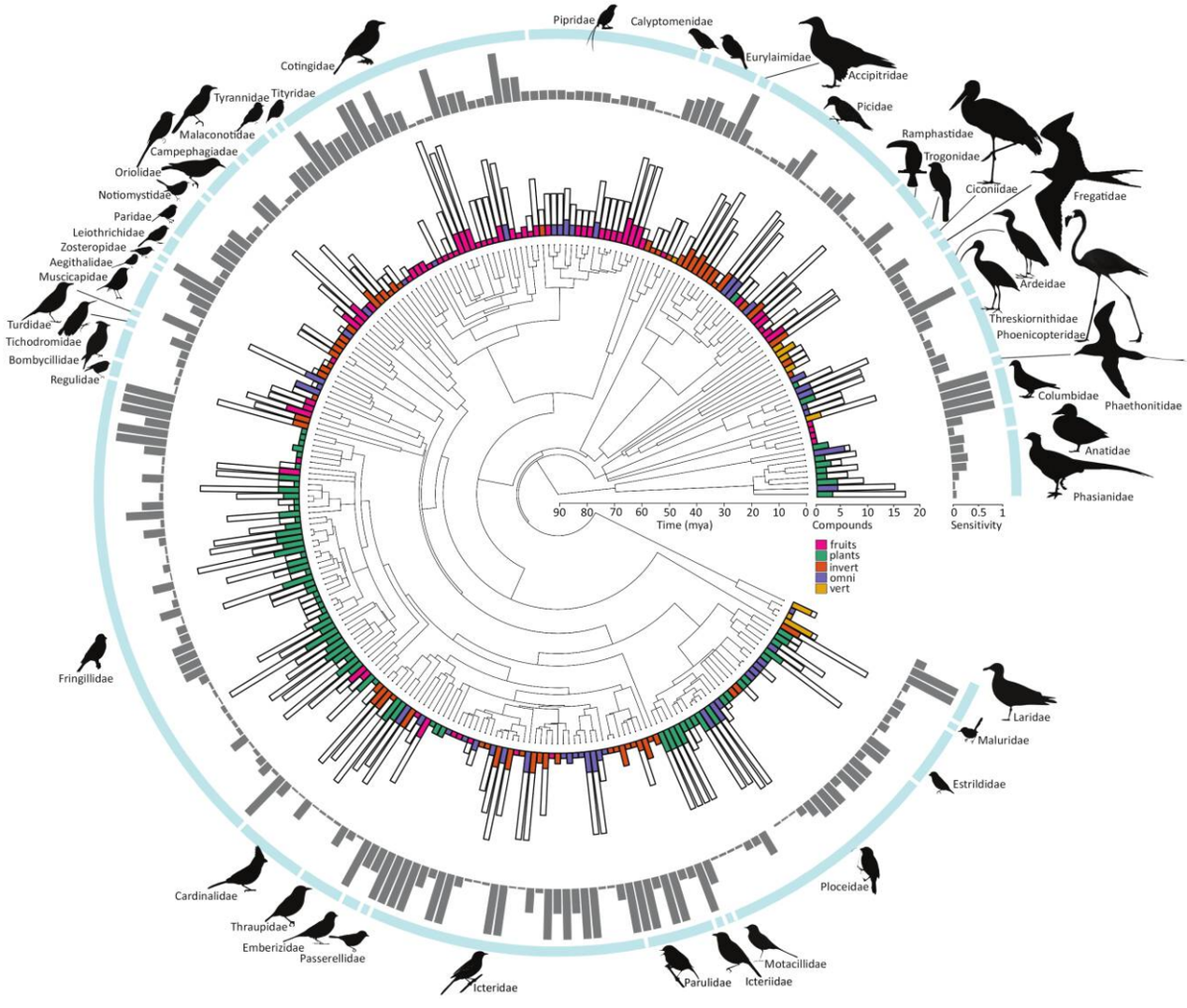

Figure 2: Evolution of resilience and ecological specialization in avian carotenoid networks. Shown are the phylogenetic distribution of diet, carotenoid network structure, and network sensitivity across 259 species of 45 taxonomic families (light blue outer ring) spanning 90 million years of avian evolution (fig. S1; table S1; supplementary data S1, S2). Overall network size (number of carotenoids) is partitioned into dietary carotenoids (colored according to the ecological classification) and metabolized carotenoids (white bars) synthesized by enzymatic conversion of dietary carotenoids (fig. S2). Gray bars show network sensitivity. Data for the figure: table S1; supplementary data S3, S4; supplementary code S1, S4. mya = million years ago.

Phylogeny, Ancestral Reconstructions, and Independent Contrasts

We constructed an ultrametric 50% majority-rule consensus tree of 259 species (figs. 2, S1; supplementary data S3, S4; supplementary code S4) from 1,000 trees randomly sampled from the pseudo-posterior distribution of the Stage2 MayrAll Hackett dataset from https://birdtree.org (Jetz et al. 2014) using SumTrees version 4.4.0 (Sukumaran and Holder 2019) in DendroPy version 4.4.0 (Sukumaran and Holder 2010). While our dataset contains the carotenoid metabolic network for the red-shafted flicker (*Colaptes auratus cafer*), the subspecies was not included in the Jetz et al. (2014) phylogeny. Thus, only the carotenoid metabolic network for the northern flicker (*Colaptes auratus*) was included in phylogenetic analyses.

To construct an ancestral metabolic network at each internal node in the ultrametric consensus phylogeny and to track individual evolutionary rates of gain and loss of carotenoid compounds, unique ancestral states (present or absent) needed to be established for all of the potential compounds and reactions in the avian space of the universal metabolic network, following the protocols in Morrison and Badyaev (2018). The ancestral states for each of the 55 compounds and 91 reactions were individually estimated using joint maximum likelihood ancestral reconstruction based on the algorithm developed by Pupko et al. (2000) using the software r8s (ver. 1.81; Sanderson 2003, 2016). Two distinct continuous-time Markov models of binary trait evolution were tested for the joint reconstructions of each compound and reaction, which were considered to be discrete (present or absent) and unordered traits (Marazzi et al. 2012). In the one-parameter model (binary-1), the rates at which a compound or reaction was gained (r, transition from absent to present) or lost (s, transition from present to absent) were equal in an instantaneous rate matrix, whereas the instantaneous rate matrix in the two-parameter model (binary-2) had distinct gain and loss rates. The Markov model with the lowest Akaike information criterion was used in the joint reconstruction of the ancestral states and the corresponding rates of gain and loss (state changes per million years) for each compound and reaction across the phylogeny (tables S3, S4).

In joint reconstruction, the ancestral states are the single set of character states across the internal nodes of the phylogeny that maximize the likelihood of observing the extant species data on the tips of the phylogeny based on the joint posterior distributions of ancestral character states on the phylogeny (Pupko et al. 2000; Yang 2006; Revell and Harmon 2022). As a result, only one character state is assigned to each of the internal nodes in the phylogeny, and there is no measure of uncertainty in this model. The decision to use joint reconstruction instead of marginal reconstruction, which would have included a measure of uncertainty for each ancestral state at internal nodes, was based on the need to construct an ancestral metabolic network at each internal node of the phylogeny from the independent reconstructions of each of the compounds and reactions. Furthermore, the most likely joint reconstruction for a set of character states usually aligns with the most probable character state in marginal reconstructions at each internal node (Pupko et al. 2000; Yang 2006; Gascuel and Steel 2014).

The ancestral network for each internal node in the phylogeny was composed of all of the compounds and reactions that were present at that node in their respective ancestral reconstructions (tables S3, S4). In cases where an internal node had an absent reaction that would be necessary to produce a present metabolized compound, that reaction was added in. Similarly, if a reaction was present at an internal node but the compound was not, this compound was added. The ancestral reconstructions of the networks therefore represent the maximum potential size (in terms of the total number of compounds and reactions) that each of the networks could be to accurately represent the biochemical properties of carotenoid metabolism. Importantly, these additions to the ancestral networks impact only overall network measures. They do not affect the rates of gain or loss of individual compounds and reactions, which are directly measured during the individual ancestral reconstruction of each network element. Comparisons of evolutionary rates of gain and loss of dietary, derived, and degenerate compounds (table S4) were used to test variation in the lability of carotenoid types during avian evolution using a least significant difference test and by calculating the coefficient of variation (CV) of the difference between the rates of gain and loss for each type of compounds. To examine the evolutionary changes in the relative prevalence of each carotenoid type, the proportions of dietary, derived, and degenerate compounds out of the total number of compounds in the network were calculated for all ancestral networks across 11 time periods within the last 20 million years of avian evolution (table S4). Time periods were selected to maximize the number of available reconstructed networks in each one. Phylogenetically independent linear contrasts were calculated with ape version 5.4 (Paradis et al. 2004) in R version 4.0.1 (R Core Team 2020) on the consensus phylogeny with randomly resolved polytomies (table S2; supplementary code S3).

Network Resilience and Distributed Robustness Measures

We used three measures to assess robustness of each species' enzymatic network. The sensitivity (ξ) of a network to the loss of individual compounds is defined as the average fraction of compounds that can no longer be produced

out of the total in the network when any compound is removed (adapted from Ebenhöh et al. 2005). At $\xi = 0$, the network is completely robust; the deletion of any node in the network has no effect on the production of any of the other compounds in the network. At $\xi = 1$, the network is maximally sensitive, and the removal of any compound leads to network disappearance. The resilience of a network to loss of a compound is $1 - \xi$.

To quantify the global enzymatic interconnectivity between all of the compounds in the a species' network (i.e., species interactome), we used a modified Shannon diversity index (after Zitnik et al. 2019). In a network with N compounds, with k isolated components $\{C_1, C_2, ..., C_k\}$ of groups of carotenoids that are connected to each other via enzymatic reactions but are not connected to carotenoids in other groups, the modified Shannon diversity index (H_{msh}) was

$$H_{msh} = -\frac{1}{\log N}\sum_{i=1}^{k} p_i \log p_i,$$

where $p_i = |C_i|/N$ is the fraction of carotenoids in the species' network that are in an isolated dietary carotenoid–specific biochemical module C_i. Thus, the Shannon diversity index measures the extent of fragmentation of the enzymatic pathways in a network; when $H_{msh} = 0$, all compounds in the network are connected via enzymatic pathways, and when $H_{msh} = 1$, all compounds are isolated dietary compounds (table S1).

We used the CV of diameter and biochemical scope starting with dietary compounds within each species' network as a measure of distributed robustness and propensity to form degenerate nodes at the intersection of diet-specific biochemical pathways. For dietary compounds, the diameter was the shortest distance (in reactions) starting with each of the dietary compounds (e.g., lutein, zeaxanthin, β-carotene, β-cryptoxanthin) and the most distant derived carotenoid metabolically reachable from this dietary compound in each species' network (details in fig. S2; tables S1, S3, S4). Biochemical scope is a measure of chemical connectivity of the network and is the number of derived compounds that can be synthesized from each of the dietary starting points in a species' network (Handorf et al. 2005; Badyaev et al. 2015).

Results

Dietary Specialization Is Associated with Lesser Network Resilience

For all species in the dataset, we characterized the ecological category and the size, structure, and resilience of carotenoid enzymatic networks (table S1; supplementary data S1, S2). We find high variability of interactomes within taxonomic and ecological groups: closely related or ecologically similar species routinely differ in the structure of their carotenoid networks, while distantly related species often converge (fig. 2). This pattern is expected from repeated subsampling and recombination of diet-specific metabolic modules within the limited and interconnected avian space of the global carotenoid network. The frequency of this recombination and the associated evolutionary cycles of occupancy of the avian space are reflected in wide fluctuations in network sensitivity within taxonomic families and ecological groups (fig. 2). However, across all ecological groups and network sizes, networks with isolated diet-specific modules were less resilient, whereas those with the same number of dietary and synthesized compounds but more interconnected networks were more resilient (fig. 3).

Stable Dietary Specialization Favors Biochemical Redundancy and Degeneracy

In birds or any taxa with obligatory dependence on exogenous inputs for carotenoid production, fragmentation of enzymatic networks can be mitigated only by merging diet-specific biochemical modules (fig. 1*B*). The more upstream (closer to the dietary component; smaller L in fig. 1*B*) this merging occurs, the more resilient the resulting network will be, because more of the network will be retained when any dietary compounds disappear. To directly measure the effect of such merging on network resilience, we used two metrics of the biochemical reach of dietary compounds—dietary diameter and biochemical scope. Species with more similar biochemical reach of distinct dietary carotenoids have more resilient networks (fig. 4*A*, 4*B*); however, ecological groups differ in this similarity. Omnivorous species, which consume a diversity of widely distributed dietary carotenoids (table S1; fig. S2), have the most similar biochemical reach of these compounds in their networks, whereas frugivorous species, which often consume unique or rare dietary carotenoids that could require distinct biochemical conversion, have the highest variation in biochemical scope and diameter of their dietary carotenoids (fig. 4*C*, 4*D*).

Degenerate Compounds Sustain Continuity of Evolution

The dual requirement of reduced fragmentation (fig. 3) and dietary interchangeability (fig. 4*A*, 4*B*) identifies degenerate carotenoids as key determinants of network resilience. We thus examined the relative contribution of degenerate compounds to the evolutionary persistence of and transitions between extant and ancestral carotenoid networks (tables S3, S4). We find that degenerate carotenoids had twice longer persistence and three times lesser evolutionary lability than derived carotenoids (all three

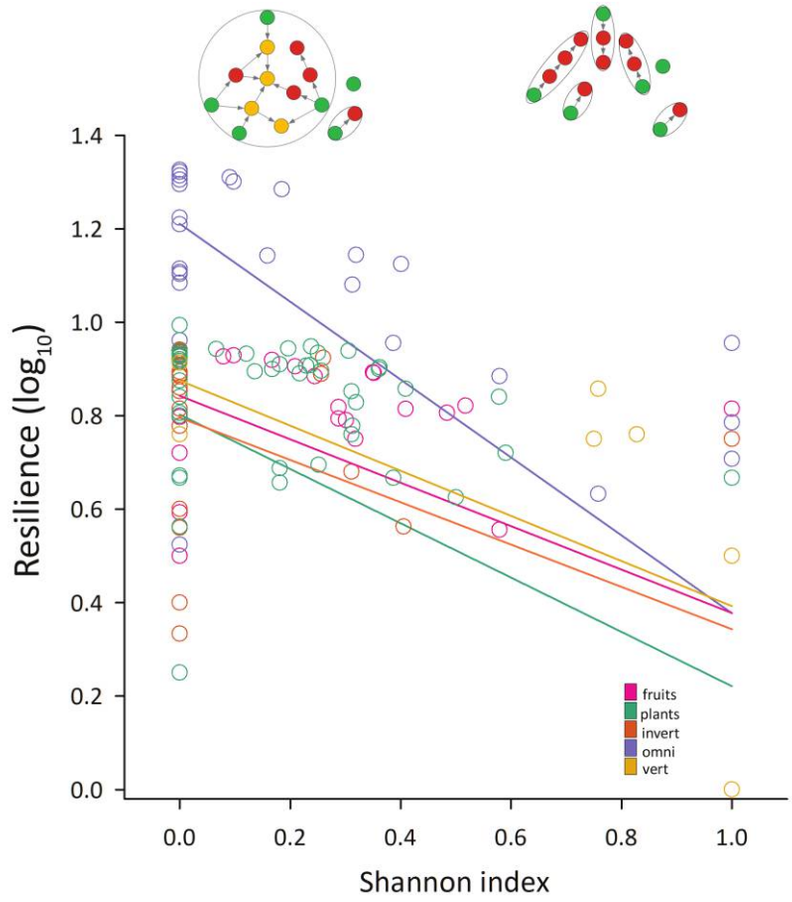

Figure 3: Less fragmented biochemical networks are more resilient across all ecological groups ($b_{ST} = -0.77$ to 0.62, $P < .001$ for all; ecological groups do not differ: $F_{4, 259} = 1.45$, $P = .22$). Lesser fragmentation is assured by degenerate compounds linking diet-specific modules. Insets show schematic examples of species' networks with the same number of nodes but low (*left*) and high (*right*) Shannon index of enzymatic fragmentation. Green circles indicate dietary carotenoids, red circles indicate derived carotenoids, and yellow circles indicate degenerate carotenoids.

carotenoid groups—dietary, derived, and degenerate—differed from each other; gains: all $|t| > 3.26$ for all, $P < .01$ for all; losses: $|t| > 8.03$ for all, $P < .001$ for all; fig. 5*A*). Very high rates of evolutionary losses of dietary carotenoids and the associated losses of carotenoids derived from them explain the overall sensitivity of avian carotenoid networks to the loss of individual compounds (fig. 2).

We then examined the relative prevalence of dietary, nondegenerate derived, and degenerate carotenoids over the last 20 million years of avian evolution (tables S2, S4). We find that degenerate carotenoids are more prevalent than derived nondegenerate carotenoids (including those linking dietary and degenerate nodes; fig. 1*A*) across all examined time periods (all three carotenoid groups differed from each other; $|t| > 4.72$ for all, $P < 10^{-4}$ for all; fig. 5*B*). Taken together, these results suggest that network resilience is enhanced by early and upstream merging of diet-specific modules, where most metabolic elaboration and branching occurs downstream of degenerate nodes (fig. 1*B*). Thus, interactome degeneracy sustains both metabolic elaboration and ecological specialization in species' networks (fig. 5*D*). This scenario is further corroborated by the finding that metabolic degeneracy, and not only the acquisition of additional dietary compounds (i.e., dietary redundancy or a wider dietary niche), maintains the enzymatic connectivity of avian carotenoid networks over time (fig. 5*C*).

Discussion

These analyses reveal that carotenoid enzymatic interactomes vary with ecological specialization and identify a mechanistic link between the maintenance of local

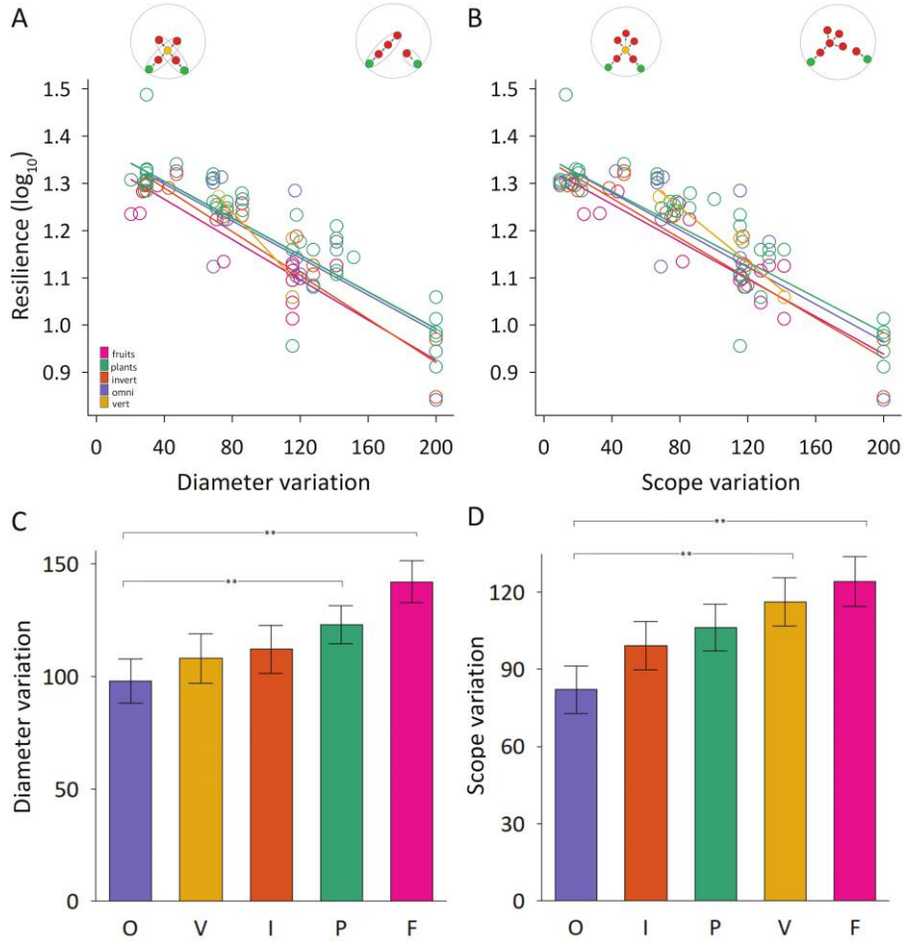

Figure 4: Dietary specialization is associated with lesser network resilience. Networks with more uniform biochemical reach across their dietary precursors are more resilient: *A*, diameter variation of individual dietary carotenoids in a species' network (coefficient of variation [CV], %; $b_{ST} = -0.89$ to 0.79, $P < .0001$ for all; ecological groups do not differ: $F_{4,259} = 1.64$, $P = .23$; *B*, biochemical scope variation of individual dietary carotenoids in a species' network (CV; $b_{ST} = -0.90$ to 0.81, $P < .0001$ for all; ecological groups do not differ: $F_{4,259} = 1.68$, $P = .16$). *C*, *D*, Ecological groups (specializing in fruits [F], plants [P], invertebrates [I], omnivorous [O], and vertebrates [V]) differ in uniformity of biochemical reach across dietary carotenoids within a species' network: it is most similar in omnivorous species and most distinct in frugivorous species. Brackets connect significantly different groups ($t_{crit} > 1.97$, $P < .05$, least significant difference test). Insets show schematics of species network with low (*left*) and high (*right*) variation in diameter (*A*) and biochemical scope (*B*) of dietary carotenoids.

specializations and transitions between them. Specifically, we show that degeneracy of avian carotenoid networks can reconcile the competing demands of robustness, which is required for local function, and evolvability, which is needed for continuous evolution (Edelman and Gally 2001; Whitacre and Bender 2010; Friedlander et al. 2017).

We identified similarity in the biochemical capacity of dietary carotenoids as the main driver of network resilience (fig. 4). This similarity can be formalized as the ratio of external to internal controls of a network that drives metabolite propagation (Ruths and Ruths 2014; Badyaev 2019). Specifically, when two or more dietary (i.e., external) biochemical pathways merge at an intermediate node, this merging is followed by exploration of the downstream enzymatic network now shielded from environmental fluctuations of each dietary precursor. When outgoing enzymatic connectivity of the intermediate node exceeds the range of dietary pathways merging into it, this node requires additional internal control to distribute flux among downstream pathways (e.g., Kacser and Burns 1973). This sustained exploration of downstream connectivity is essential for diversification and elaboration of carotenoid-based traits in taxa that are fully dependent on exogenous carotenoids. For example, in birds it underlies such well-known

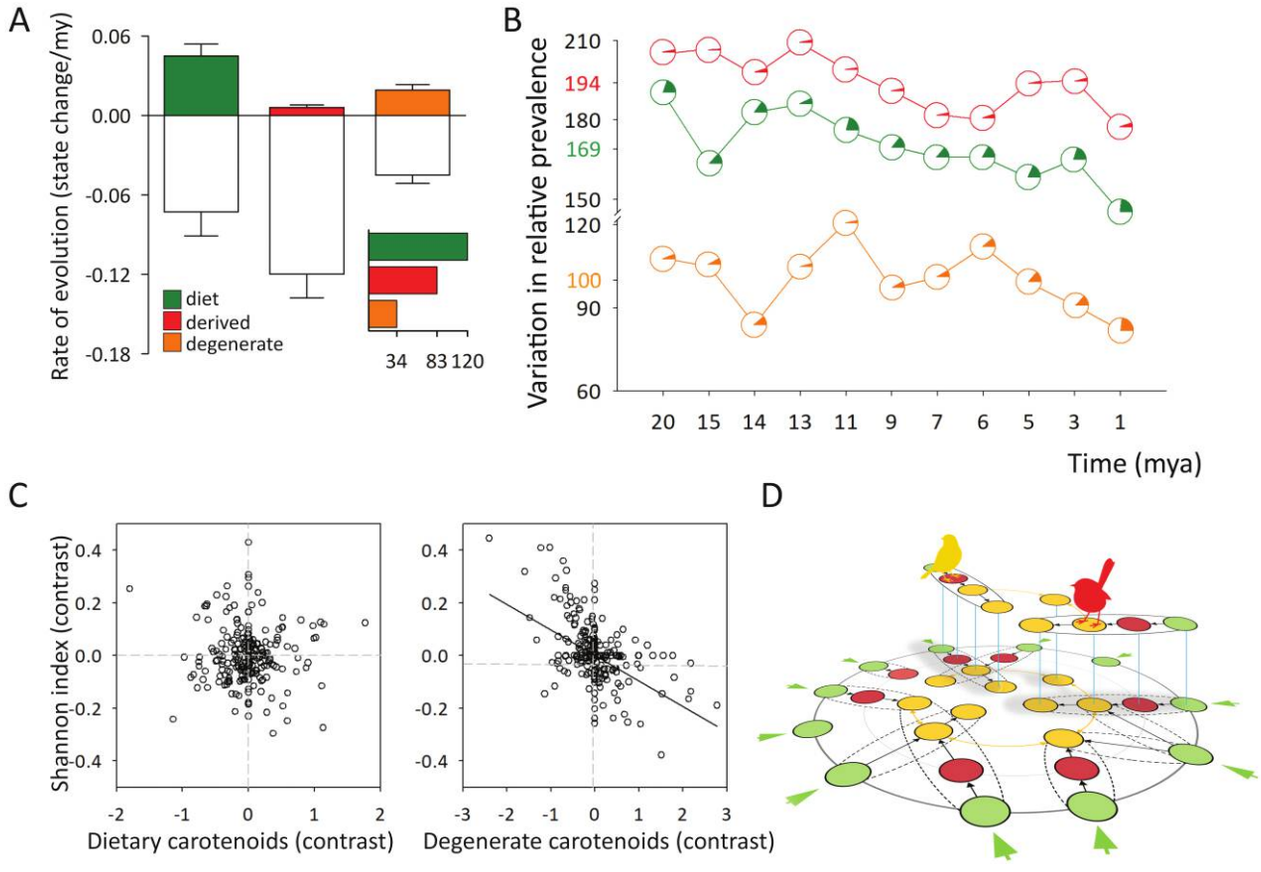

Figure 5: Degenerate compounds sustain continuity of avian carotenoid evolution. *A*, Frequency of gains (above *x*-axis) and losses (below *x*-axis; state changes/million years, mean ± SD) for dietary, derived nondegenerate, and degenerate carotenoids. Degenerate carotenoids have a lower rate of evolutionary losses than nondegenerate carotenoids, whereas dietary carotenoids have the highest rate of evolutionary gains, which is similar to the rate of their evolutionary losses. The inset shows the coefficient of variation (CV; %) of evolutionary lability (difference between losses and gains) for the three classes of carotenoids. *B*, Variation (CV) in the relative prevalence of dietary, derived, and degenerate carotenoids (with respect to the total number of compounds in the network) in ancestrally reconstructed networks. Average CV for each group across all time periods is shown in the corresponding font color on the *y*-axis. Pie charts show mean prevalence of a carotenoid type in all networks of ancestral taxa present during each time period. Degenerate carotenoids are more reliably present than either their dietary or derived precursors across all time periods. mya = million years ago. *C*, Only degenerate carotenoids contribute to uninterrupted network connectivity over evolutionary time. Shown are partial regressions of independent linear contrasts (dietary: $b_{ST} = -0.05$, $t = -0.68$, $P = .49$; degenerate: $b_{ST} = -0.51$, $t = -9.55$, $P < .001$; $F_{2,54} = 6.16$, $P = .01$). *D*, Conceptual summary of results. Degenerate compounds enable transitions between specializations, link adaptations with evolutionary diversifications, and sustain exploration of biochemical connectivity from different dietary entry points.

macroevolutionary patterns as the recurrent convergence of distantly related and ecologically distinct taxa in carotenoid pigments of their plumage and the rapid divergence of ecologically similar species in carotenoid biosynthesis. Gain of internal controls in avian carotenoid networks not only enables more resilient local adaptation but also harbors greater diversification potential, whereas loss of internal controls constrains carotenoid evolution to the rate of the gains and losses of dietary precursors (Badyaev et al. 2015, 2019).

Importantly, the patterns of avian exploration of the global carotenoid network, such as variation in the acquisition of degenerate carotenoids by different ecological groups found here (fig. 5; fig. S2), are not determined by the structure of the network itself. Randomization approaches showed that the avian subsampling of the global carotenoid network is strongly constrained by the lability of the most upstream (dietary) carotenoids, such that any sustainable evolution of carotenoid-based ornamentation requires periodic acquisition of degenerate carotenoids (Badyaev et al. 2015, 2019). High lability of source dietary carotenoids also precludes direct transitions between specialized networks in the absence of degenerate nodes as stepping stones (fig. 5*D*). In contrast, in randomized explorations

of the global carotenoid network, connectivity of subnetworks is always accomplished by the elongation of biochemical pathways, which is not observed in real-world evolution (Badyaev et al. 2019). Correspondingly, historical cycles of transitions between external and internal controls of carotenoid networks underlie the tempo and mode of carotenoid network evolution in birds and in other taxa where this mechanism has been explicitly investigated (Borenstein et al. 2008; Badyaev et al. 2019).

Much higher evolutionary gains and losses of dietary carotenoids compared with remarkably stable degenerate nodes across a diversity of dietary precursors (figs. 2, S2), as well as the greater contribution of degenerate nodes to the connectivity of carotenoid interactomes (fig. 5*C*), are most consistent with the ecology-to-history scenario under which recurrent merging of diet-specific biochemical modules underlies the evolutionary persistence of degenerate carotenoids (fig. 1*B*). This scenario is corroborated by the observation that the gains and losses of new degenerate compounds follow the gains and losses of distinct dietary biochemical modules (Badyaev et al. 2019) and are not associated with the retention of the preceding degenerate compounds, as predicted by the preadaptation scenario (fig. 1*B*).

Dietary carotenoids, which identify ecological specialization of a group (Gray 1996; Olson and Owens 2005; McGraw 2006; fig. 2), alone do not account for either the long-term stability of a metabolic network or its evolutionary persistence (fig. 5). Instead, the branching pattern that exists in the biochemical vicinity of these dietary carotenoids is key: when this branching enables the formation of degenerate nodes, it facilitates both local specialization and evolutionary diversification (fig. 5*D*). On the contrary, ecological taxa with limited connectivity in the biochemical vicinity of dietary carotenoids will have limited opportunity for sustained evolution of carotenoid elaboration. This pattern gives us a mechanistic null expectation for expected carotenoid-based ornamentation in avian groups, including in relation to their ecological specialization. An important caveat, however, is that species' carotenoid networks are typically larger than networks used solely in integument pigmentation because carotenoids are used in a multitude of additional organismal functions (e.g., Twyman et al. 2016; Toomey and Corbo 2017). In fact, such retention of larger carotenoid networks is thought to underlie evolutionary subsampling and recombination of metabolic motifs involved in integument pigmentation, including within species (Morrison and Badyaev 2017, 2018). Furthermore, this conclusion pertains only to biochemical derivation of carotenoids and not their optical properties resulting from variable integration with integument.

These findings illustrate three evolutionary principles. First, greater evolutionary importance of highly interconnected enzymes comes not from their specialization but from their role in maintaining network robustness (e.g., Vitkup et al. 2006). Elements of locally essential specialized processes are often replaced, whereas degenerate elements involved in numerous functional pathways or controlling variable metabolic fluxes have longer evolutionary persistence (e.g., Rausher 2012; Rancati et al. 2018). Second, in addition to greater compensation for fluctuations in dietary inputs, degenerate compounds, by definition, distribute the production of the same compounds across several pathways (Morrison and Badyaev 2016*b*, 2017). This distribution and compensatory interactions between degenerate pathways modify selection acting on each one, leading to drift of intermediate enzymatic pathways (Vaishnav et al. 2022) and thereby increasing diversification of their end products (Aharoni et al. 2005). Third, involvement in structural maintenance versus local functions enables degenerative compounds to transit to a new function without losing the original one, thereby enabling a direct transition from one specialization to another without passing through intermediate stages, mechanism duplication, or a generalist stage (Khersonsky and Tawfik 2010). Thus, although degenerate nodes structurally bridge sequential adaptations, they do not retain the ecological specialization of preceding adaptive states and thus facilitate evolutionary diversification. Instead, degenerate nodes reflect the strength, stability, and overlap between sequential adaptations (e.g., Sorrells et al. 2015)—that is, evolutionary contingency.

In sum, by enabling access to biochemical connectivity and ultimately maintaining the cohesion of biochemical networks (fig. 5*D*), degenerate compounds act as springboards for subsequent evolutionary diversification while at the same time constantly updating evolutionary trajectories to match with the prevalent ecological context.

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Statement of Authorship

A.V.B. and E.S.M. designed the project and obtained funding, E.S.M. and C.M.H. collected data, E.S.M. and A.V.B. analyzed data, A.V.B. wrote the original draft, and all authors reviewed and edited the final version.

Data and Code Availability

Data and code for this article are in the Harvard Dataverse (https://doi.org/10.7910/DVN/ES36IN; Morrison et al. 2025).

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Saguaro pollen provides an important food source for house finches in the Sonoran Desert, with pigments contributing to the finch carotenoid-based coloration. Photo by Alex Badyaev|tenbestphotos.com.