RESEARCH ARTICLE

Developmental Integration of Feather Growth and Pigmentation and its Implications for the Evolution of Diet-Derived Coloration

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ABSTRACT

Variation in avian coloration is produced by coordinated pigmentation of thousands of growing feathers that vary in shape and size. Although the functional consequences of avian coloration are frequently studied, little is known about its developmental basis, and, specifically, the rules that link feather growth to pigment uptake and synthesis. Here, we combine biochemical, modeling, and morphometric techniques to examine the developmental basis of feather pigmentation in house finches (*Carpodacus mexicanus*)—a species with extensive variation in both growth dynamics of ornamental feathers and their carotenoid pigmentation. We found that the rate of carotenoid uptake was constant across a wide range of feather sizes and shapes, and the relative pigmented area of feathers was independent of the total amount of deposited carotenoids. Analysis of the developmental linkage of feather growth and pigment uptake showed that the mechanisms behind partitioning the feather into pigmented and nonpigmented parts and the mechanisms regulating carotenoid uptake into growing feathers are partially independent. Carotenoid uptake strongly covaried with early elements of feather differentiation (the barb addition rate and diameter), whereas the pigmented area was most closely associated with the rate of feather growth. We suggest that strong effects of carotenoid uptake on genetically integrated mechanisms of feather growth and differentiation provide a likely route for genetic assimilation of diet-dependent coloration. *J. Exp. Zool. (Mol. Dev. Evol.) 318:59–70, 2012.* © 2011 Wiley Periodicals, Inc.

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The plumage of an individual bird is produced by the coordinated growth and pigmentation of thousands of individual feathers that vary in shape, size, and color. The interplay of feather growth and pigmentation produces tremendous diversity of within- and across-taxa coloration patterns, from elaborated sexual ornaments to exceptional camouflage matching (Lillie and Juhn, '32; Lucas and Stettenheim, '72; Burtt, '86). Although numerous studies have examined the functional consequences of avian coloration (reviewed in Baker and Parker, '79; Hill and McGraw, 2006), the developmental dynamics of avian plumage patterns remains poorly studied. Yet, contemporary functional significance of plumage variation tells us little about the proximate mechanisms behind its development and evolution; indeed, studies show that simple modulation of developmental mechanisms integrating growth and pigmentation within a feather can result in a remarkable diversity of pigmentation patterns and feather shapes (Nickerson, '44; Chuong and Edelman, '85; Prum, '99; Brush, 2000; Prum and Williamson, 2002; Yu et al., 2002;

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Harris et al., 2005; Bortolotti et al., 2006), such that similar sexual ornaments can be produced by a variety of developmental mechanisms. Although feathers can only be colored during growth, few studies have investigated the developmental linkage between feather growth and pigment uptake (Prum and Williamson, 2002; Badyaev and Landeen, 2007). Yet, such knowledge is crucial because it gives us powerful insight into the proximate mechanisms behind ecological and evolutionary diversification of plumage coloration.

Such investigation is particularly important for dietary-derived pigments, such as carotenoids. It is often suggested that developmental processes associated with carotenoid-based coloration can indicate individual condition and health (Rothschild, '75; Endler, '83; Hoelzer, '89; Hill, 2006). In birds, such condition dependence is thought to be produced by variable developmental integration of processes involved in acquisition, metabolism, and transport of diet-dependent carotenoids allocated to integument coloration with other organismal processes (Badyaev and Young, 2004; Badyaev, 2007). Allocation to plumage depends crucially on an interaction between carotenoid supply delivered to each feather follicle and dynamics of feather growth, and several studies pointed out that carotenoid uptake affects general morphology of ornamental feathers, such as the presence of finely differentiated barbules (Olson, '70; Troy and Brush, '83; Bleiweiss, 2004), whereas direct investigation of developmental integration of feather growth and carotenoid pigmentation revealed that several elements of feather growth are crucial determinants of feather pigmentation (Badyaev and Landeen, 2007). However, it is not known what developmental components of feathers are modified by carotenoid uptake or even whether coloring of feathers vary with the amount of pigment available at the time of feather growth, e.g., whether feather growth can compensate for pigment type and availability (Fig. 1).

Here, we examine the developmental basis of within-feather pigmentation in male house finches (*Carpodacus mexicanus*), a species in which both growth dynamics of ornamental feathers and their carotenoid pigmentation have strong fitness consequences (Hill, 2003; Badyaev and Vleck, 2007). In house finches, as in most birds, carotenoids color the distal (the oldest) and middle parts of the feather, suggesting that growth of an ornamented feather starts only when pigments are already available in the follicle (Fig. 1A, scenario i). Alternatively, feather growth and uptake of carotenoids can be triggered and terminated by a particular concentration of carotenoids within a follicle (Fig. 1A (ii and iii) and B). These scenarios can be inferred from a relationship between the amount of pigment taken by a growing feather and the relative area of the feather that is colored by this pigment and from structural modifications of feathers required for carotenoid uptake.

First, we took advantage of extensive variation in size, shape, and pigmentation of ornamental feathers to deduce general rules by which variation in growth and carotenoid deposition contributes to feather coloration. Second, we use a biologically informed model of feather growth to identify ontogenetic mechanisms responsible for partitioning a feather into pigmented and nonpigmented parts and regulating carotenoid uptake. Third, we examine covariation between these mechanisms and discuss its implication for ornament elaboration and evolutionary diversification of carotenoid-based plumage.

MATERIALS AND METHODS

Study Population and General Methods

Feather samples for this study ($n = 363$ feathers from 121 ornamental parts of 41 males, 10 from yellow morph, and 31 from red morph) were obtained in 2004–2006 from 2 year old male house finches in the southwestern Arizona study population (description of the study site and general protocols are in Badyaev and Vleck, 2007). All resident birds were individually marked in this population and age and molt status was known for all birds included in this study. All males included in this study were captured within a month of the completion of postbreeding molt, and 15 ornamental feathers (5 from each of the three ornamental areas: crown, breast and rump; Fig. 1 in Badyaev and Landeen, 2007) were taken from each bird.

Feathers were digitized using a modified Epson Perfection 1660 Photo scanner (Long Beach, CA) at 1,000 dpi. In each feather, we digitized nine landmarks whose variation is closely associated with known components of feather growth (after Badyaev and Landeen, 2007). The landmarks were (Fig. 1 in Badyaev and Landeen, 2007; and Fig. 5A) (1) base of feather calamus, (2) base of feather rachis, (3) structural change boundary along rachis, (4) end of rachis, (5) tip of feather, (6, 7) widest part of feather, and (8, 9) boundary of isochronic angle formed by (3). Landmark coordinates were acquired with tps software (SUNY Stony Brook, F.J. Rohlf) and rachii curvatures were standardized for all feathers with tpsUtil software (SUNY Stony Brook, F.J. Rohlf; see Badyaev and Landeen, 2007, for protocol). Total feather area (all landmarks) and the pigmented area of a feather (landmarks 3, 5, 6, 7, 8, and 9) were calculated as centroids of perimeter landmarks (see Procrustes superimposition methods below). For each feather, we measured intensity of coloration (degree of white reflectance of a pixel) by sampling one pixel per barb in the pigmented area of the feather with SigmaScan Pro 5.0 (SPSS, Inc., New York) and averaging the values across all barbs ($n = 28–73$ barbs per feather). Methods for hue intensity measures, adapted for measuring a single feather, are in Badyaev and Duckworth (2003).

Modeling Feather Growth

We modeled feather growth with Mathematica 5.2 software (Wolfram Research, Inc. 1988–2005) following a general six-parameter model of feather growth proposed by Prum and Williamson (2001) and modified for ornamental feathers by

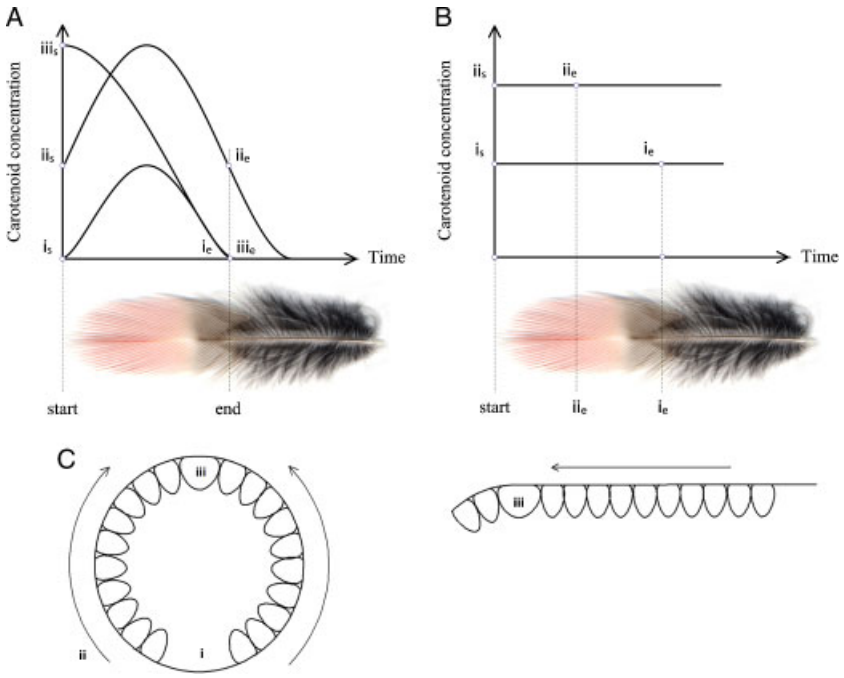

Figure 1. Schematic illustration of developmentally informed scenarios by which amount of carotenoids can influence the pigmented area of a feather. The distal tip of the feather is the beginning of feather growth and pigmentation (*s*), the end of pigment deposition is marked by (*e*). (**A**) Concentration of carotenoids in follicle leads to variable pigmented area of a feather. Scenario (i)—feather is growing only when carotenoids are present, so that duration of uptake coincides with the amount of time carotenoids are present in the feather follicle; scenario (ii)—carotenoid uptake and feather modification are triggered and terminated by a particular concentration of carotenoids within a follicle; scenario (iii)—carotenoid uptake is initiated by a particular concentration of carotenoids within a follicle (maximum here) and lasts until all carotenoids are deposited. (**B**) Pigmented area is regulated by *the total amount of carotenoids* allocated in the feather, e.g., scenario (i)—lower concentration of carotenoids is associated with larger pigmented area (and lower concentration within a feather) and scenario (ii)—higher concentration with the smaller pigmented area (and higher concentration within a feather). Independence of pigmented area and carotenoid concentration and availability suggests that feather allometry and feather modifications enabling carotenoid uptake are developmentally decoupled. (**C**) Cross-sectional view of feather follicle. During feather growth, new barbs are formed at locus (i) at the posterior portion of the follicle and (ii) migrate toward the anterior end where they fuse to create the rachis (iii). "Unfurled" feather follicle was used in growth simulations where simulated feathers grow along the linear *x*-axis.

Badyaev and Landeen (2007). The model is based on six parameters, each corresponding to a particular element of feather growth (after Prum and Williamson, 2001; Harris et al., 2005): (1) absolute growth rate, m, (2) angle of barb growth, θ, (3) initial number of barb ridges, n, (4) rate of addition of new barbs, B, (5) barb diameter, α, and (6) angle of expansion of the ramus, β. The model simulates the size and position of the barbs and rachis, and the total size of the feather follicle during the growth of a pennaceous feather (Fig. 1F in Badyaev and Landeen 2007). Barb growth was simulated using two matrices of the x and y coordinates; one followed the coordinates of the barb tips and the other followed the coordinates of the barb bases within a feather. Growth was modeled as a series of consecutive time steps; for each time step, the new coordinates of the barb bases and tips were updated in the matrices.

In brief, absolute growth rate, m, described the rate at which the rachis and the barbs grew per time step and remained constant throughout growth. The angle of barb growth, θ, described the angle between the barbs and rachis. During growth, the feather follicle had an initial number of barbs, n, and grew new barbs, determined by a ridge addition function, $B(t)$. Because feather growth ends when all barbs are fused to the rachis, the rate of addition of barbs is less than that of their fusion. Following Prum and Williamson (2001), we used a linearly decreasing function for rate of addition of barbs:

$$B(t) = -\frac{w}{20} + w + 1,$$

where 20 is the number of time steps over which barbs are added and w determines how much the equation varies from one, i.e.,

the slope of the linearly decreasing rate of addition of barbs varies with w. The follicle of a growing feather is not fixed in diameter (Harris et al., 2005), but varies with the number and diameter of barbs. Thus, the diameter of barb ridges was described by the function:

$$d(t) = d_{max} - (d_{max} - d_0)e^{(t-t_0)/\alpha}$$

that approaches d_{max}, the maximum ridge diameter; t_0 is the time step at which the barb emerges, d_0 is the initial radius of the barb; and α is the rate at which the barb reaches d_{max}. In feathers with constant diameter, the diameter is d_{max}. For simulations reported here, the initial diameter of the barb, d_0, did not vary, but the rate of increase in diameter was allowed to vary through the adjustment of α. Angle of expansion of the barb β occurs after the barb's emergence from the feather sheath, with expansion of the ramus forcing the barbs to expand outward from the rachis.

To simulate feather growth, we "unfurled" the circular follicle so that feathers grew along the linear x-axis (Fig. 1C). Following the Prum and Williamson (2001) model, we began simulations of growth with the emergence of the initial barb ridges in the follicle, with new barbs being added at the new barb loci on the posterior end of the follicle. Barb ridges grew at rate m, migrating toward the anterior end of the follicle; the anterior-most initial ridges then met and fused, formed the rachis, and continued to grow at rate m. As each barb reached the rachis it fused, completing its growth, and continued to migrate upward with vertical growth of the rachis. The follicle diameter varied with the number and diameter of the barbs present in the follicle at a given time. Simulated feather growth ceased when all barbs present in the follicle had completely fused to the rachis and the barbs unfurled by the expansion angle, β. To create predicted patterns of movement of the landmarks, each of the six model parameters were manipulated individually while keeping others statistically constant. Parameters were modeled in ten steps each: feather growth (m) in ~0.2 increments from 0.60 to 2.50, barb growth angle (θ) in ~5° increments from 15 to 70°, initial number of barbs (n) in increments of 2–4 from 8 to 36; rate of addition of barbs (w) in increment of 0.16 from 0.40 to 2.20, barb–ridge diameter (α) in increments of ~0.11 from 0 to 1.15, and expansion angle of the barbs (β) in increments of 5° from 0 to 45° (Badyaev and Landeen, 2007).

Feather Carotenoid Pigment Extraction

Carotenoids were extracted and identified from each feather using high-performance liquid chromatography (HPLC). Pigmented portion of each feather was cut, weighed (0.001–3.7 mg, depending on the ornament part), washed in hexane, and dried in a vacuum pump using Whatman GF/A glass filters. After washing, feathers were finely ground in 3 mL methanol for 10 min at 20 Hz using a Retsch MM301 mixer mill (Newtown, PA), equipped with ZrO grinding jars and balls. Carotenoids were then extracted using a 0.2 μm filter (GHP Arcodisc 13 mm Minispike; Pall Life Sciences, East Hills, NY), and the filtrate was evaporated to dryness under vacuum at 40°C and reconstituted in 100–200 μL HPLC mobile phase (50:50 v/v methanol:acetonitrile).

Carotenoids were quantified by injecting 50 μL of mobile phase containing pigment extract into an HPLC System (Shimadzu Corporation, Pleasanton, CA) fitted with an YMC Carotenoid 5.0 μm column (250 × 4.6 mm) and guard column (Waters Corporation, Milford, MA). Analytes were eluted at a constant flow rate of 1.2 mL/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 returned with step function to the initial isocratic condition at which it was held through 48 min. Carotenoids were detected using a Shimadzu SPD-M10AVP photodiode array detector, and data were collected from 200 to 800 nm. Peaks and concentrations per unit of feather mass (ug/g) were calculated using calibration curves of standards (Sigma, St. Louis, MO; Indofine Chemical, Hillsborough NJ; CaroteNature, Lupsingen, Switzerland); peak areas were integrated at 450 or 470 nm depending on the absorbance maximum (λ_{max}) for each compound. All compounds were combined for this study to calculate a total allocation of carotenoids for each feather.

Data Analysis

To analyze the biologically informed ontogenetic transformation in pigmented feather structure (see *Modeling feather growth* above) across a range of shapes and sizes, we applied a Procrustes superimposition (Rohlf and Slice, '90; Klingenberg and McIntyre, '98) to align the landmark configurations of fully grown feathers from different ornaments, carotenoid uptakes, pigmented areas, individuals, and individual replicas. Variance in the set of optimally aligned landmark configurations (hereafter, Procrustes coordinates) was then partitioned using ANOVA models (Goodall, '91; Badyaev and Foresman, 2000). Degrees of freedom for the Procrustes ANOVA were calculated following Goodall ('91) and Klingenberg and McIntyre ('98). To partition the effects of each landmark on overall variation in feather shape, we first summed x and y Mean Squares of each landmark and computed variance components for total amount of carotenoids, pigmented area, body part, and individual variation according to the expected Mean Square for each of the effects (after Badyaev and Foresman, 2000). We analyzed the covariance matrices of the Procrustes coordinates and, based on the expected Mean Squares, computed separate matrices of Sums of Squares and Cross-products for each of the effects (Table 1).

To visualize patterns of covariation in the landmarks owing to each effect, we graphically represented principal components (PCs) of each of the matrices as displacement of landmarks from

Table 1. Variance components (% variance) for displacement of ornamental landmarks (left side of feather only) owing to the effects in the Procrustes ANOVA of feather shape

	Effects									
	Carotenoid		Pigmented area		Body part		Individual		Error	
Ornamental landmark	Variance	%	Variance	%	Variance	%	Variance	%	Variance	%
3	0.002	21.7	0.004	43.9	0.003	34.0	0	0	4.1	0.4
5	0.004	66.9	0.001	19.0	0.0005	8.4	0.0003	5.0	4.0E−05	0.8
6	0.005	68.0	1.2E−05	0.2	0.002	28.7	0.0001	2.2	5.7	1.1
8	0.017	77.0	5.8E−05	0.3	0.003	12.3	0.002	9.5	0.0002	0.9

Underlined values are significantly different from zero at $\alpha = 0.05$.

their consensus position. The vector associated with each landmark represented the direction and magnitude of displacement of this landmark owing to an effect. To examine similarity between patterns of landmark covariation within and between observed and simulated samples, we computed the angles between the first PCs as $= \arccos [a'b/(a'ab'b)^{0.5}]$ where a and b are the eigenvectors to be compared. Statistical significance and distribution of angles for comparison of observed and predicted vectors were obtained by resampling of the within-sample PC coefficients for each effect separately (dashed lines in Fig. 6). We used nonparametric two-tailed Kruskal–Wallis tests and general linear model to provide descriptive statistics for all other comparisons.

RESULTS

Morphology and Pigmentation of Ornamental Feathers

Feathers from different ornament areas differed strongly in overall size (Fig. 2A; $\chi^2 = 106.4$, $P < 0.001$), size of pigmented area (Fig. 3A; $\chi^2 = 87.8$, $P < 0.001$), and the total amount of deposited carotenoids (Fig. 3A; $\chi^2 = 12.6$, $P = 0.002$). Crown feathers had more saturated color (i.e., lower intensity of hue) than feathers from other ornamental parts (Fig. 2B; $\chi^2 = 55.6$, $P < 0.001$). The percentage of pigmented area did not differ between crown and breast feathers (Fig. 3B; $\chi^2 = 1.7$, $P = 0.35$) despite their large size difference and was smaller for rump feathers (Fig. 3B). Carotenoid concentration (per g of feather) did not differ across feather types (Fig. 3B; $\chi^2 = 1.0$, $P = 0.58$). Total amount of carotenoids in a feather did not correlate with the size of pigmented area of a feather in either of the three feather types (Fig. 3C; crown: $b_{ST} = 0.14$, $t = 0.90$, $P = 0.37$, breast: $b_{ST} = -0.11$, $t = 0.68$, $P = 0.51$, rump: $b_{ST} = 0.19$, $t = 1.18$, $P = 0.29$). Across all ornamental feather types, feather color intensity varied with the total amount of carotenoids allocated to the feather, but did not vary with the pigmented area of feather (Fig. 4A–C).

Modification of Feather Structure and Shape by Carotenoid Allocation and Growth

Joint assessment of the effects of total amount of carotenoids, pigmented area partitioning, body part, and individual identity showed that carotenoid uptake had an overwhelming effect on feather structure, shape, and differentiation (>58% of variation averaged across all landmarks), followed by effects of body part (20.9%), pigmented area partitioning (15.9%), and individual identity (2.5%; Table 1). Modifications of feather structure due to variation in carotenoid uptake (Fig. 5A) were most similar to those caused by follicular changes in barb diameter ($r_v = 0.59$), the rate of barb addition during early feather differentiation ($r_v = 0.51$), and absolute growth rate ($r_v = 0.44$). Variation caused by changes in pigmented area of a feather (Fig. 5B) was mostly due to changes in feather growth rate ($r_v = 0.61$), barb diameter ($r_v = 0.57$), and the angle of helical growth (vertical projection of absolute growth rate) ($r_v = 0.54$). Structural changes in feathers on different body parts were indistinguishable from the effects of the initial barb number ($r_v = 0.82$) and the angle of expansion once the feather emerged from the skin ($r_v = 0.72$). Individual identity effects were weak and significantly similar only with variation in absolute growth rate ($r_v = 0.39$). Overall, total uptake of carotenoids and partitioning of pigmented feather area had similar effect on feather structure and shape ($r_v = 0.78$, ($\gamma = 38.7^\circ$)), despite partially distinct developmental elements that they affected (Fig. 5A and B).

DISCUSSION

Evolution of integument coloration requires close association of pigment uptake, integument growth, and modification. Numerous studies have addressed the functional significance and evolutionary diversification of avian coloration (reviewed in Hill and McGraw, 2006), yet the developmental integration between feather growth and pigment uptake (presumably the proximate target of selection producing adaptation and diversification in avian colors) is unstudied. This is particularly true for

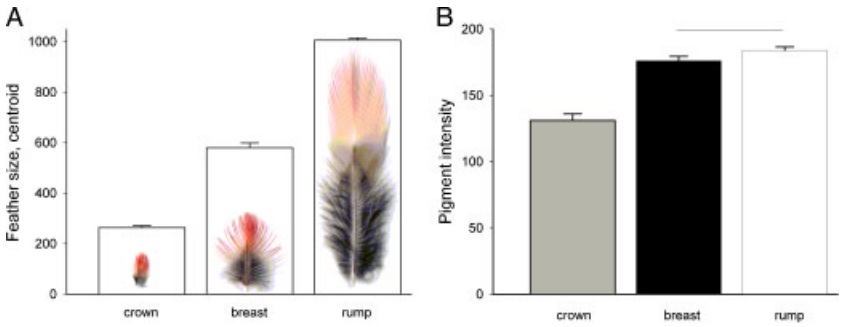

Figure 2. Mean ± s.e.m. of (A) feather size and (B) intensity of pigmentation in the three ornamented areas of house finch plumage. Greater intensity indicates lower color saturation. Line connects bars that do not differ from each other.

diet-derived carotenoid coloration, where an external origin of feather pigments necessitates integration of acquisition, synthesis, and transport of carotenoids to the feather follicle with synthesis of keratin in a growing feather. Ontogenetically, feather is a product of activation–inhibition cycle of keratinogenesis (Harris et al., 2005; Obinata and Akimoto, 2005; Yue et al., 2005, 2006), and until recently we lacked a biologically informed model that would interpolate developmental variation in key elements of three-dimensional feather growth into size and shape of fully grown two-dimensional feather. Such a geometric transformation model was developed by Prum and Williamson (2001), who showed that *in silico* modifications of six growth parameters is sufficient to produce most of the observed diversity of feather shapes (see also Chuong and Edelman, '85; Chuong, '93; Jiang et al., '99). Recently, we extended this model to the study of ornamental feathers (Badyaev and Landeen, 2007) and showed that *in silico* modifications of the same growth parameters also adequately describe observed within-species variation in carotenoid-derived feather pigmentation across populations, ages, and color morphs. The important result of that study was that within-follicle integration of feather growth and pigmentation plays a defining role in the evolution of diet-derived pigmentation patterns. However, the nature of this integration has not been studied directly.

Here, we combined biochemical, morphometric, and modeling approaches to show that carotenoid acquisition is closely associated with feather growth and structure. There were three main results. First, concentration of deposited carotenoids per unit of feather mass was similar across feathers of variable sizes and shapes, indicating that carotenoid uptake by a growing feather were largely constant during growth. Second, the proximal boundary of feather area that can be pigmented was determined by the mechanisms that were partially independent of the total amount of carotenoids available for deposition, such that there was no relationship between the size of pigmented area and the amount of carotenoids deposited into a feather. Consequently, variation in feather saturation was due to variation in total pigment uptake and did not vary with area of the feather that was pigmented (e.g., there were no enhancing or compensating effects of area in relation to pigment amount). Third, we identified specific developmental elements most affected by incorporation of carotenoids into the feather and demonstrated partial independence of within-follicle processes regulating growth and coloration of feathers.

Carotenoid-bearing ornamental feathers commonly have a modified structure (typically less secondary differentiation, e.g., barbule absence or fusion (Brush and Seifried, '68; Olson, '70; Troy and Brush, '83; Hudon, '91; Blanco et al., 2005; Shawkey and Hill, 2005). However, whether such modification is a cause or a consequence of carotenoid deposition and which developmental elements of feather growth are most affected was not known. Here, we show that carotenoid uptake is closely associated with modification of early elements of feather differentiation: the barb addition rate and diameter (Fig. 5A). Such modifications resulted in significant changes in width and elongation of the fully grown feather (e.g., more than 65% of variation in landmarks 5, 6, 8 was caused by variation in carotenoid uptake), the angle of feather expansion, and absolute growth rate (Fig. 6A). Such early developmental changes of barb number and diameter suggests that feather structure needs to be significantly modified to enable carotenoid uptake, such that ornamental feather growth and carotenoid synthesis have to coincide or the presence of carotenoids should trigger ornamental feather growth (Fig. 1). The finding of similar feather carotenoid concentrations among widely distinct feather types (Fig. 3B) further suggests that the initial growth of ornamental feathers does not proceed until there is an adequate supply of carotenoid pigments in the feather follicle or circulating plasma. In this house finch population, ornamental feathers do not have a nonpigmented fringe at the distal and lateral parts of feathers, the earliest growing parts, suggesting that initiation of growth and carotenoid uptake coincide (such fringe is sometimes present in

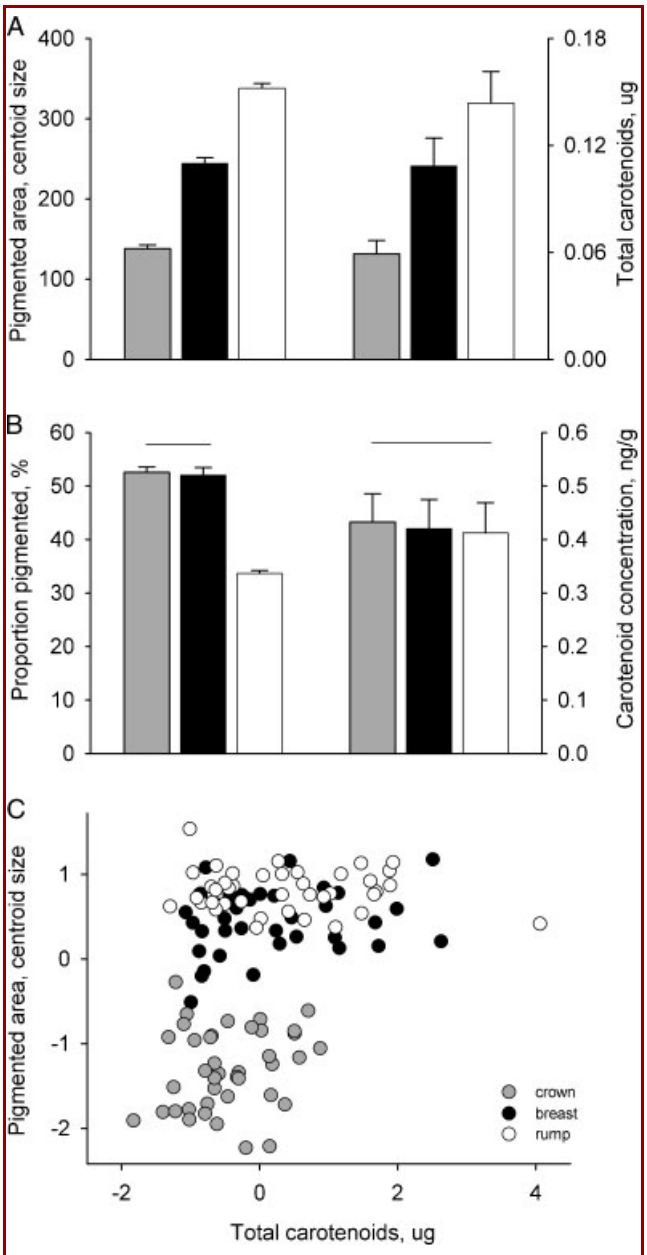

Figure 3. Variation across the three ornamental feather types (crown: gray, breast: black, and rump: white) in (A) pigmented area and total amount of carotenoids, (B) proportion of feather pigmented and carotenoid concentration (per *g* of feather), and (C) relationship between standardized (mean = 0, s.d. = 1) values of total carotenoid amount deposited into each feather and pigmented area of that feather. Line connects bars that do not differ from each other.

northern populations of house finches where it is worn off during the winter preceding breeding). At the same time, the proximal boundary of the pigmented area is always clearly delineated (Fig. 1) and forms a pronounced transition in structural differentiation between the distal (pigmented) and proximal (unpigmented) part of the feather. This suggests that the scenario ii in Figure 1A or B is most likely, such as carotenoids below a certain concentration are not incorporated by a growing feather.

Across species, the deposition of carotenoids into the feather follicle varies from passive lipid diffusion (Voiketvich, '66; Lucas and Stettenheim, '72) to the formation of variable bonds with feather keratin (Desselberger, '30; Hudon and Brush, '89; Bleiweiss, 2004; Blanco et al., 2005; Shawkey and Hill, 2005). Passive lipid-enabled diffusion of carotenoids produces patterns of coloration that are chiefly influenced by two factors: the type of carotenoid pigment present in the follicle (Brush and Power, '76; Inouye et al., 2001; McGraw et al., 2006) and modification of feather growth that enable this pigment to be deposited (Badyaev and Landeen, 2007). Despite significant variation among the three ornamental feather types in size, shape, and intensity of color (Figs. 2 and 3), the concentration of carotenoid per unit of feather mass was similar, suggesting that the keratinocytes that make up the feather barbs and incorporate carotenoid pigments are constrained by the total mass of carotenoids they can uptake, regardless of the specific carotenoid compound. These results corroborate previous findings that, in house finches, differences in feather hue are largely caused by variation in the proportions of specific carotenoid compounds and not by variation in the absolute carotenoid mass per feather (Brush and Power,'76; Inouye et al., 2001; McGraw et al., 2006). Although here we focused on the effect of the total amount of carotenoid uptake, preliminary results suggest that the effect of carotenoid pigments on feather microstructure is related to the molecular weight of particular carotenoid compounds—a pattern likely caused by the size of follicular pores or constraints of barb diameter (A.V. Badyaev et al., unpublished manuscript).

Interestingly, the developmental mechanism that strongly correlates with the pigmented area in a feather is the same that causes age-related variation in feather growth and pigmentation (Badyaev and Landeen, 2007). In at least two house finch populations, older males have a greater pigmented proportion of ornamented feathers and grow these feathers at a faster rate compared with yearling males, proximately accounting for both the faster molt and greater postmolt ornamentation of older males (Badyaev and Vleck, 2007). Elsewhere, we showed that a single developmental parameter—angle of helical growth of feather (horizontal projection of cell addition rate in each barb)—is sufficient to account for most of the age-related variation in feather shape and pigmentation proportions (Fig. 6 in Badyaev and Landeen, 2007). An important finding of this study is that this is the only developmental parameter *unaffected* by the amount of carotenoid uptake during feather growth (Fig. 6A; *p*2) further corroborating developmental uncoupling of feather partitioning into pigmented and unpigmented parts and uptake of carotenoids during growth.

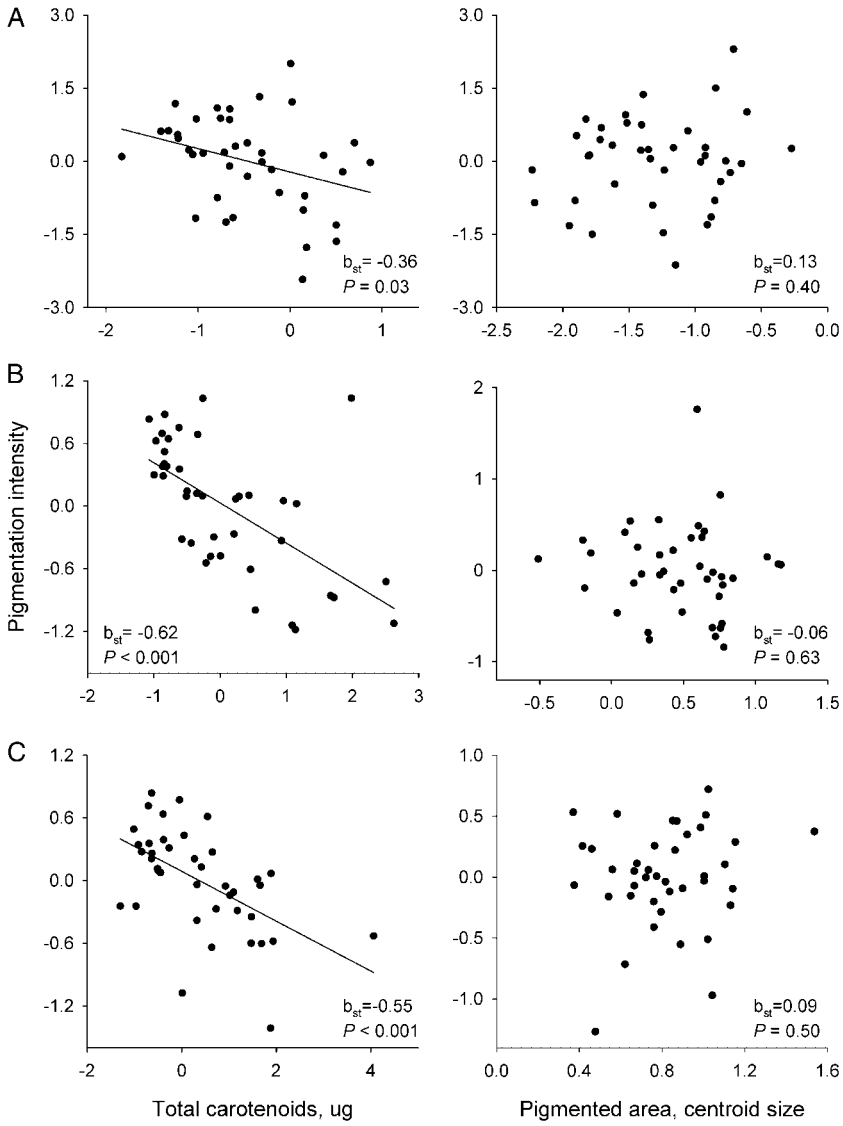

Figure 4. Relative contribution of total carotenoid allocation and pigmented area to feather pigmentation intensity in (A) crown, (B) breast, and (C) rump ornamental feathers. Shown is partial regression plots, b_{st} is a standardized regression coefficient (in s.d.). Greater intensity indicates lower color saturation (i.e., greater white reflectance).

If the amount of carotenoids in the feather follicle is proportional to organism-wide circulation of carotenoid precursors during molt, then the finding of passive uptake of pigments by a growing feather has important implications for evolution of optimal molt strategies. In finches, molt of different ornaments commonly overlaps and the degree of overlap affects overall elaboration of ornamentation (Badyaev and Vleck, 2007). Because feathers on different pigmented areas have different areas to be colored (Fig. 3), the expenditure of organism-wide carotenoids will vary across ornamental areas, suggesting that there is an optimal amount of overlap between feather molt in these areas in relationship to availability of carotenoids during molt.

Joint examination of developmental variation in ornamental feathers (Fig. 5) revealed a hierarchical organization of ornamental plumage where location within a particular ornamented body part determines gross morphology of the feather follicle, such as its diameter and initial number of barbs (see also Badyaev and Landeen, 2007 for similar results), the onset of carotenoid deposition modifies within-feather structure during early phases of feather growth, temporarily changing barb addition rate and diameter, whereas the partially independent rate of keratinogenesis,

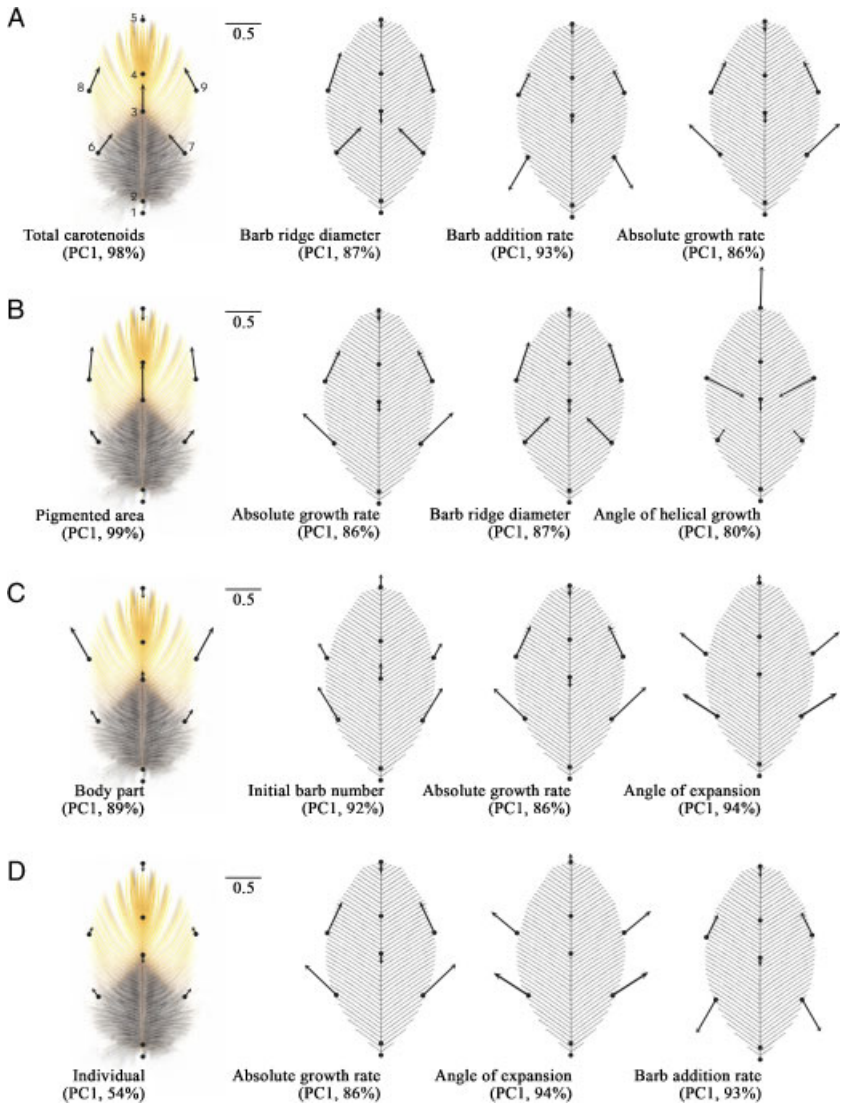

Figure 5. Observed and predicted patterns of structure variation in fully grown ornamental feathers owing to variation in (**A**) total amount of carotenoid uptake during growth, (**B**) pigmented area of feather, (**C**) ornamental part (body part), and (**D**) individual identity. Left column shows *observed* landmark displacements due to each effect, three right columns show in silico ontogenetic modifications that generate closest predicted landmark displacement to the observed for each effect (see Fig. 6 and text). Principal component (PC) loadings from Procrustes superimposition are shown as vectors originating at the consensus configuration for each landmark, with vectors delineating the magnitude (vector length) and direction of PC coefficients. Numbers are percent of variation accounted for by PC1 of the procrustes mean squares for each source of variation. Vector length of 0.5 loading is shown for scale. All landmarks were used in calculations, but only the displacements of landmarks delineating ornamental part of the feather are shown.

which determines absolute growth rate and angle of helical growth, determines the relative area of a feather that can be pigmented.

That carotenoid uptake modifies fundamental aspects of feather growth provides a crucial insight into the evolution of carotenoid-based ornamentation (Badyaev, 2007; Badyaev and Landeen, 2007). First, the feather follicle is a highly modular structure, where several autonomous physicochemical emergent processes are regulated by conserved genetic circuitry of activation–inhibition effects (Chuong and Edelman, '85; Chuong et al., '90; Chuong, '93; Harris et al., 2005). Such organization enables a feather follicle to produce an exceptional diversity of sizes and shapes not only across species but also across the lifetime of an individual bird (Prum and Brush, 2002; Bartels,

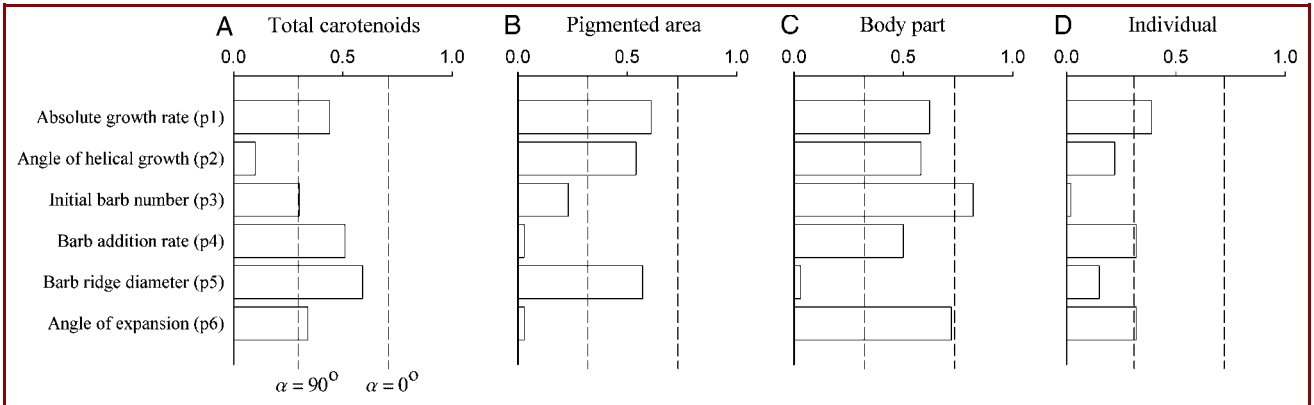

Figure 6. Concordance between predicted and observed ornamental–landmark displacement (Fig. 5) for feather structure modification owing to **(A)** total uptake of carotenoids during growth, **(B)** pigmented area of a feather, **(C)** ornamental (body) part, and **(D)** individual identity. Shown are vector correlations between PC1 of procrustes Mean Squares of observed sample and PC1 of predicted set. Correlations to the right of the right-most dashed line indicate the vector angles not different from 0° (i.e., statistical concordance), correlations between the two dashed lines indicate angles significantly different from both 0 and 90°, correlations to the left of the left-most dashed line indicate angles not different from 90° (i.e., complete dissimilarity).

2003; Prum, 2005; Badyaev and Landeen, 2007). Second, close integration of diet-derived pigment and genetically modular regulation of feather growth is likely accomplished by a common involvement of several key proteins that regulate both, keratin synthesis during feather growth and carotenoid transport to the follicle (Badyaev, 2007). Foremost among these is prolactin or a prolactin mimic that is not only synthesized in response to epidermal invasion by a growing feather and regulates feather growth and diversification, but also plays an important role in lipid and lipoprotein transport in plasma (Rose et al., '95; Barron et al., '99; Gossage et al., 2000; Dawson, 2006; Badyaev and Vleck, 2007). Such co-option of shared developmental regulators provides a likely route for genetic assimilation of diet-derived pigmentation. Pituitary prolactin involvement in regulation of parental care (Vleck et al., 2000; Badyaev and Vleck, 2007), often signaled by carotenoid-based ornaments (Duckworth et al., 2003), should further enhance the developmental entrenchment of diet-derived pigments and facilitate their evolutionary retention.

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