

# Developmental evolution of sexual ornamentation: model and a test of feather growth and pigmentation

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**Synopsis** A tremendous diversity of avian color displays has stimulated numerous studies of natural and sexual selection. Yet, the developmental mechanisms that produce such diversification, and thus the proximate targets of selection pressures, are rarely addressed and poorly understood. In particular, because feathers are colored during growth, the dynamics of feather growth play a deterministic role in the variation in ornamentation. No study to date, however, has addressed the contribution of feather growth to the expression of carotenoid-based ornamentation. Here, we examine the developmental basis of variation in ornamental feather shapes in male house finches (*Carpodacus mexicanus*)—a species in which carotenoid displays are under strong natural and sexual selection. First, we use geometric morphometrics to partition the observed shape variation in fully grown feathers among populations, ages, degrees of elaboration, ornamental body parts, and individuals. Second, we use a biologically informed mathematical model of feather growth to predict variation in shape of ornamental feathers due to simulated growth rate, angle of helical growth of feather barbs, initial number of barb ridges, rate of addition of new barbs, barb diameter, and ramus-expansion angle. We find close concordance between among-individual variation in feather shape and hue of entire ornament, and show that this concordance can be attributed to a shared mechanism—growth rate of feather barbs. Predicted differences in feather shape due to rate of addition of barbs and helical angle of feather growth explained observed variation in ornamental area both among individuals and between populations, whereas differences in helical angle of growth and the number of barbs in the feather follicle explained differences in feather shape between ornamental parts and among males of different ages. The findings of a close association of feather growth dynamics and overall ornamentation identify the proximate targets of selection for elaboration of sexual displays. Moreover, the close association of feather growth and pigmentation not only can reinforce condition-dependence in color displays, but can also enable phenotypic and genetic accommodation of novel pigments into plumage displays providing a mechanism for the observed concordance of within-population developmental processes and between-population diversification of color displays.

## Introduction

A fundamental goal of evolutionary theory is understanding the link between the causes of individual variation and the causes of variation among generations and taxa (Chetverikov 1926; Huxley 1942; Mayr 1942). A central empirical question arising from this goal is the extent to which the commonly studied causes of organismal *differences* can be attributed to the causes of organismal *production* (Lewontin 1983; Oyama et al. 2001). Despite major recent advances in evolutionary ecology and developmental biology, however, the link between the origin and diversification is unknown for most organismal forms (Müller and Newman 2003; West-Eberhard 2003; Orr 2005; Young and Badyaev 2007).

There are few systems in which this gap is more evident than in studies of avian color displays—some of the most extravagant and diverse traits in the living world. On the one hand, there is a multitude of studies of natural and sexual selection on avian coloration stimulated by the tremendous diversity of avian displays and their functions (reviewed by Baker and Parker 1979; Burt 1986; Badyaev and Hill 2003; Hill and McGraw 2006). On the other hand, there is a nearly complete lack of empirical studies of developmental mechanisms behind such diversification, especially at the most fundamental unit of avian plumage—the feather (Price et al. 1991; Prum and Brush 2002; Bartels 2003). Feathers are colored during growth (Voitkevich 1966; Lucas and Stettenheim 1972), and, whereas development of

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feathers themselves is well understood (Yu et al. 2004; Lin et al. 2006), the ontogenetic basis of feather pigmentation, with a few notable exceptions (Lillie and Juhn 1932; Nickerson 1944; Prum and Williamson 2002; Bortolotti et al. 2006), is rarely addressed. This lack of studies is particularly evident in the ontogeny of diet-derived carotenoid displays in birds. Despite significant advances in studies of metabolism of carotenoid precursors in such displays (Fox 1976; Brush 1990; McGraw 2006), to our knowledge, no study to date has addressed the mechanism for developmental integration of carotenoid coloration and feather growth.

The lack of developmental perspective in studies of avian displays has left a number of unresolved questions. First, recent developmental studies uncovered a remarkable combination of environmental sensitivity, plasticity, and developmental modularity in the cellular and molecular mechanisms of feather growth (Rouzankina et al. 2004; Yu et al. 2004; Harris et al. 2005; Kim et al. 2005; Yue et al. 2005; Lin et al. 2006), and it is thought that this enables diversification in the shapes and coloration of feathers (Harris et al. 2002; Eames and Schneider 2005). It is unclear, however, how to reconcile the remarkable developmental lability of feathers with precise patterns of coloration of camouflage plumage or with elaborated sexual displays. Second, carotenoids in avian coloration have to be obtained from the diet (Brush 1978; Britton 1998) and environments vary extensively in availability of carotenoid precursors. It is unclear how to reconcile such environmental contingency in the biosynthesis of carotenoids with the close integration of feather growth and feather coloration evident in the evolutionary diversification, elaboration, and convergence of carotenoid-based plumage displays (Badyaev 2006, 2007). A prerequisite for answering these questions, and the focus of this study, is the understanding of developmental interactions between feather growth and pigmentation.

Here, we examine the developmental basis of variation in the shape of ornamental feathers in the house finch (*Carpodacus mexicanus*)—a species in which overall carotenoid displays are under strong natural (Badyaev et al. 2001) and sexual (Hill 2003) selection. First, we examine sources of variation in ornamental coloration of house finches among populations, ages, and individuals, and explore the ways in which variation in shape and size of ornamental feathers contribute to such variation. Second, we take advantage of a biologically informed mathematical model of the diversification of feather shape proposed by Prum and Williamson (2001) to

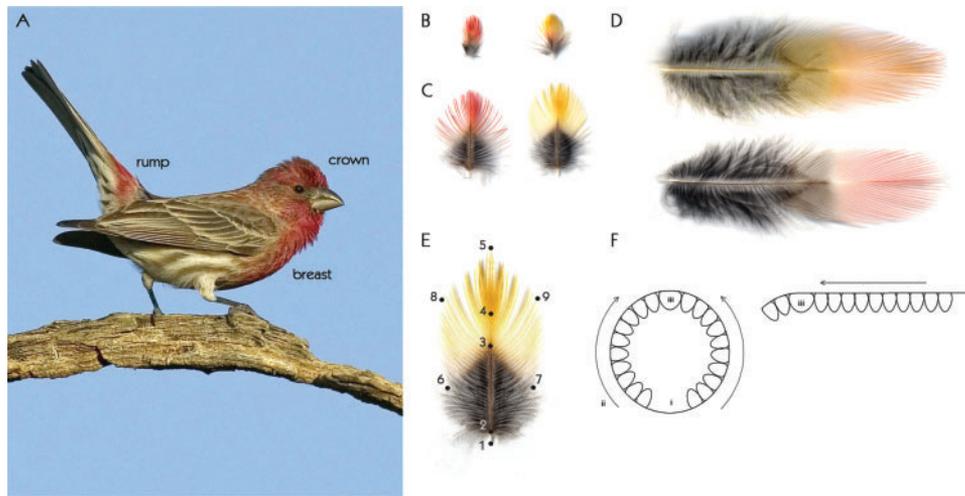
simulate growth and pigmentation patterns of ornamental feathers. We compare the patterns of ontogenetic transformation in ornamental feathers predicted by the Prum–Williamson model with those observed in the house finch to deduce the effects of growth in the feather follicle on overall elaboration and diversification of plumage. We find close integration between the dynamics of feather growth and pigmentation in carotenoid-based ornamental plumage, as well as close concordance of within-population developmental processes and between-population divergence in ornaments. We discuss the implications of these findings for understanding the links among development, function, and evolution of sexual displays.

## Materials and methods

### Study populations and general methods

House finches under this study were sampled in 2004–2006 in two populations in northwestern Montana ( $n=37$  birds, 111 ornament samples) and in southwestern Arizona ( $n=80$  birds, 240 ornament samples). In both populations, all resident birds were trapped year-around and marked with a unique combination of four rings; age was known for all birds included in this study (protocols in Badyaev and Duckworth 2003; Badyaev and Vleck 2007 for Montana and Arizona, correspondingly). After post-breeding molt, resident males were captured with stationary traps, and the ornaments of the crown, breast, and rump were photographed (Fig. 1A) using a 5-megapixel digital camera outfitted with a ring flash and mounted in a standard position (for details of protocol see Badyaev et al. 2001). For each male, we measured the overall hue of the plumage by overlaying a 10- $\times$ -10-pixel grid over each ornament patch and sampling one pixel in 10 squares of the grid on left and right sides of an ornament. The total area of the ornamental patch within each of the three ornamental parts was measured by tracing the pigmented area of each ornament and calculating area (in pixels). All measurements were conducted using SigmaScan Pro 5.0. (SPSS, inc.)

Subsequent to overall measurements of the ornament, 15 fully-grown ornamental feathers (five from each of the three ornamental areas) were plucked from each individual. All feathers were digitized using Epson Perfection 1660 Photo scanner (Long Beach, CA, USA) at 1000 dpi. After the exclusion of damaged feathers, three feathers were randomly selected for each male, one for each crown, breast, and rump ornament (Fig. 1B–D). Nine landmarks were selected to describe the shape and pigmentation



**Fig. 1** Ornamental feathers of male house finches (**A**) differ in size, shape, and pigmentation. Shown are ornamental feathers of (**B**) crown, (**C**) breast, and (**D**) rump. (**E**) Nine landmarks used in this study to describe ontogenetic and static variation in size and shape of feathers. Landmarks 3-6-8-5-9-7 delineate pigmented part of each feather. During feather growth within a follicle (**F**), new barbs are formed at the new barb locus (i) at the posterior portion of the follicle (cross-sectional view), and (ii) migrate towards the anterior end of the follicle where they fuse to create (iii) the rachis. Within-feather coloration is determined by pigmentation of each individual barb ridge during growth with carotenoids delivered to the follicle by the centrally located pulp. “Unfurled” feather follicle used in growth simulations; simulated feathers (half of feather is shown) grow along the linear x-axis.

pattern of the feathers (Fig. 1E): (1) base of the calamus, (2) base of the rachis, (3) pigment boundary along the rachis, (4) end of the rachis, (5) tip of the feather, (6, 7) lower pigment boundary, and (8, 9) the widest part of the feather. Coordinates of nine landmarks were obtained for each feather using tps software (SUNY Stony Brook, F. J. Rohlf). Curvatures of the rachii were standardized for all feathers with tpsUtil software (SUNY Stony Brook, F. J. Rohlf). Feather size was calculated as a centroid of all landmarks, the barb length was the distance between landmarks 4 and 5, and isochronic angle was the angle formed by landmarks 6-3-7 at the lower boundary of the pigmented area (Fig. 1E) that are assumed to be produced at the same time, but form this angle, visible as a result of pigment deposition, at landmark 3 due to acceleration of horizontal projections of absolute growth rate of feathers (Prum and Williamson 2001).

### Modeling feather growth

We simulated feather growth with Mathematica 5.2 software (Wolfram Research Inc. 1988–2005), using a mathematical model of feather growth proposed by Prum and Williamson (2001). According to this model, the absolute growth rate of the barbs and rachis of feathers is uniform across the follicular collar (Fig. 1), such that cell division in the follicle and the angle of ramus expansion account for ontogenetic transformations in feather shape

(Prum and Williamson 2001). In the subsequent modeling of within-feather melanin patterning, Prum and Williamson (2002) showed that spatial and temporal features of feather growth determine whether individual barbs uptake an external pigment (Lucas and Stettenheim 1972; Cheng and Brush 1984). This model provided an opportunity for manipulating individual parameters of feather growth independently and to examine the complexity and redundancy of such effects on ontogenetic transformation of the shape of ornamental feathers and of overall ornamentation.

The model utilized six growth parameters: (1) absolute growth rate,  $m$ , (2) angle of barb growth,  $\theta$ , (3) initial number of barb ridges,  $n$ , (4) rate of addition of new barbs,  $B$ , (5) barb diameter,  $\alpha$ , and (6) angle of expansion of the ramus,  $\beta$ . 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). Using the model, 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. Growth was modeled as a series of consecutive time steps, and for each time step the new coordinates of the barb bases and tips were updated in the matrices. During the simulations, the nine landmarks were followed throughout growth of the feather, and, after growth was completed (see subsequently), the coordinates of the nine landmarks were recorded.

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,  $\theta$ , described the angle between the barbs and rachis. During growth, the feather follicle had an initial number of barbs,  $n$ , and also 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 must be 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. The diameter of barb ridges was described by the function:

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

(after Prum and Williamson 2001), that approaches  $d_{\max}$ , the maximum ridge diameter; where  $t_0$  is the time step at which the barb emerges,  $d_0$  is the initial radius of the barb, and  $\alpha$  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  $\alpha$ . The final parameter, angle of expansion of the barb,  $\beta$  occurs after the barb's emergence from the feather sheath, with expansion of the ramus forcing the barbs to expand outward from the rachis.

#### Landmark displacement in fully grown feathers simulated by growth parameters

Feather growth occurs in a circular feather follicle (Fig. 1F, Lucas and Stettenheim 1972; Chuong et al. 2000). To simulate feather growth, we “unfurled” the follicle so that feathers grew along the linear  $x$ -axis rather than around a circular follicle (Fig. 1F). 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 (Fig. 1F). Barb ridges grew at rate  $m$ , migrating toward the anterior end of the follicle; the anterior-most initial ridges 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,  $\beta$ . To create predicted patterns of movement of the landmarks, each of the six model parameters were manipulated individually, creating changes in feather shape unique to that parameter. Six parameters were modeled in ten steps each—feather growth ( $m$ ) in  $\sim 0.2$  increments from 0.60 to 2.50, barb growth angle ( $\theta$ ) in  $\sim 5^\circ$  increments from  $15^\circ$  to  $70^\circ$ , 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 ( $\alpha$ ) in increments of  $\sim 0.11$  from 0 to 1.15, and expansion angle of the barbs ( $\beta$ ) in increments of  $5^\circ$  from  $0^\circ$  to  $45^\circ$ .

#### Data analysis, the localization and visualization of effects

Ornamental feathers differ in shape across body parts (Fig. 1). This variation is partially due to changes in growth parameters (see subsequently), but also due to proportional changes in feather size (e.g., crown versus rump, Fig. 1C and D). Thus, to examine variation in feather shape only, we applied a single Procrustes superimposition (Rohlf and Slice 1990; Klingenberg and McIntyre 1998) to align the landmark configurations of fully-grown feathers from different populations, ages, ornament elaborations, left and right ornament sides, individuals, and individual replicas. Variance in the set of optimally aligned landmark configurations (hereafter Procrustes coordinates) was then partitioned using analysis of variance (ANOVA) models (Goodall 1991; Badyaev and Foresman 2000). Individual identity was entered as a random effect and ornamental part was nested within an individual term. Degrees of freedom for the Procrustes ANOVA were calculated following Goodall (1991) and Klingenberg and McIntyre (1998). 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 of mean squares according to the expected mean squares for each of the effects (Badyaev and Foresman 2000; Young and Badyaev 2006). We analyzed the covariance matrices of the Procrustes coordinates and, based on the expected mean

squares, computed matrices of sums of squares and cross-products for the population, age, overall hue and area, ornamental part, and individual variation.

To visualize patterns of covariation in the landmarks due to each effect, we graphically represented principal components (PC) of each of the matrices as displacement of landmarks from their consensus position. The vector associated with each landmark represents the direction and magnitude of displacement of this landmark due 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  $\gamma = \arccos [a'b/(a'b'b)^{0.5}]$ , where  $a$  and  $b$  are the eigenvectors to be compared. Statistical significance and distribution of angles for comparison of observed and predicted vectors was obtained with resampling of the within-sample PC coefficients for each effect separately. We used nonparametric two-tailed Kruskal–Wallis tests and general linear model to test variation in overall ornamentation and feather characteristics.

## Results

### Observed variation in sexual ornamentation and feather shape

Overall ornamental area was larger in males from Arizona compared to those from Montana (Table 1; Fig. 2A). Populations did not differ in ornament hue, with the exception of a significant population-by-age interaction for hue of the breast ornament (Table 1; Fig. 2B). In Montana, older males had a greater proportion of pigmented feather area on all parts of the ornament compared to young males that acquired sexual plumage for the first time (Fig. 2D). In both populations, the area of the breast ornament was the largest, followed by rump and crown (Table 1, Fig. 2A). Ornamental crown feathers, despite being the smallest in size, had

a higher proportion of pigmented area than did the larger rump and breast feathers (Table 1, Fig. 2C and D). Barb length was similar across populations and ages. Isochronic angle, however, was significantly smaller in older males in both populations and across all ornamental parts (Table 1, Fig. 2E and F).

Landmark displacements illustrated distinct sources of variation in feather shape (Table 2, Fig. 3). Landmarks 3, 6, and 7 were most strongly affected by between-population variation, landmark 5 displacement was closely associated with male's age and overall ornamental area, displacement of landmarks 6 and 7 was strongly affected by ornament hue and among-individual variation, whereas displacement of landmarks 8 and 9 was affected by ornamental parts (Table 2, Fig. 3). The landmark displacement due to population affiliation was most closely concordant with displacement due to hue elaboration (vector correlations,  $r_v$  between displacement patterns:  $r_v = 0.65 \pm 0.12$ ,  $\gamma = 49.7^\circ$ ; Fig. 3A and C), age-related variation was highly concordant with ornamental-part variation ( $r_v = 0.61 \pm 0.09$ ,  $\gamma = 52.5^\circ$ , Fig. 3B and E), and among-individual variation was highly similar to both hue elaboration ( $r_v = 0.78 \pm 0.11$ ,  $\gamma = 39.2^\circ$ , Fig. 3C and F) and, to a lesser degree, ornamental-part variation ( $r_v = 0.62 \pm 0.14$ ,  $\gamma = 51.6^\circ$ , Fig. 3E and F).

### Simulated growth of ornamental feathers

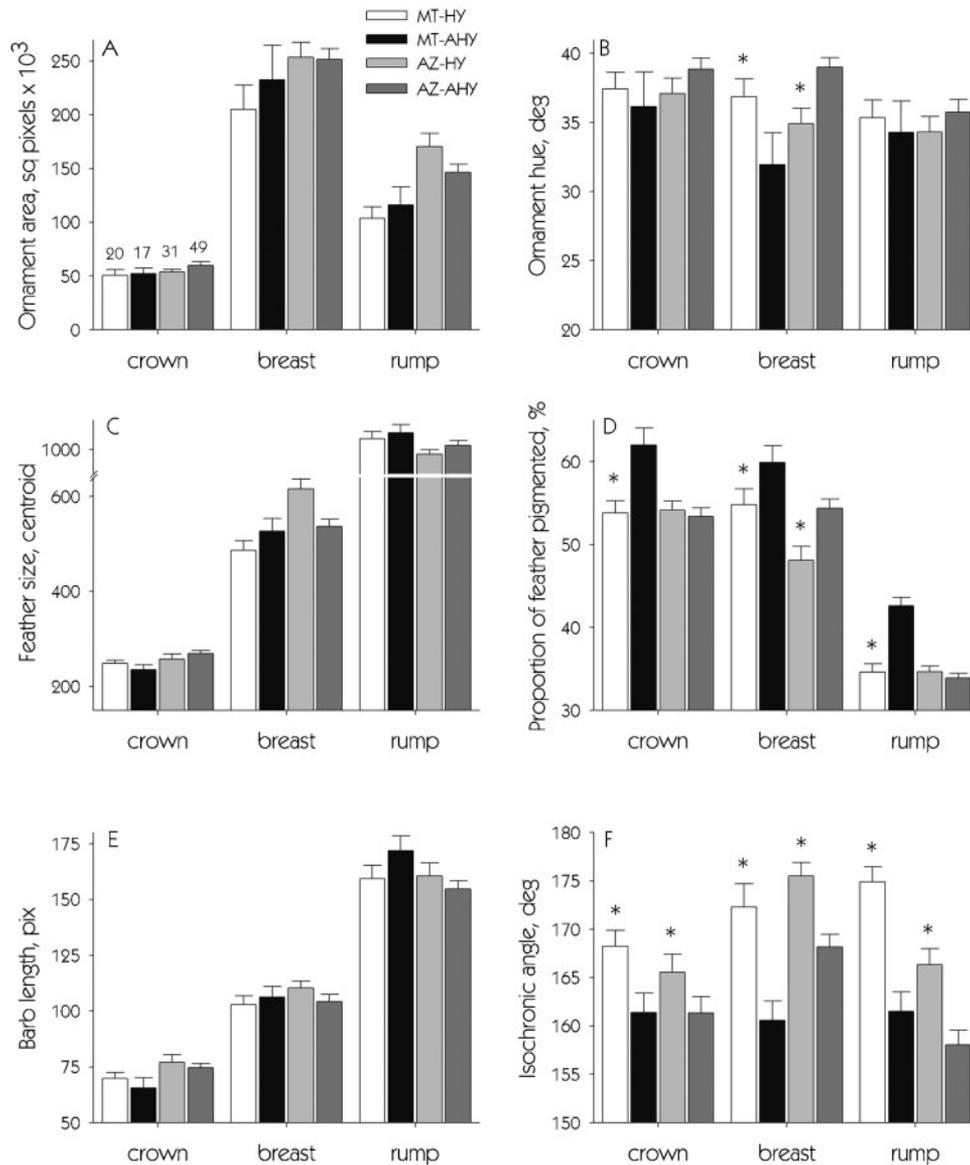
Simulation of growth parameters produced an array of feather shapes (Fig. 4) and sequential simulation of individual growth parameters resulted in distinct patterns of predicted landmark displacement (Fig. 5). The pattern of displacement due to absolute growth rate (Fig. 5A) was highly concordant with displacement due to initial number of barbs ( $r_v = 0.75$ ,  $\gamma = 41.2^\circ$ , Fig. 5C) and the ramus expansion angle ( $r_v = 0.80$ ,  $\gamma = 36.8^\circ$ , Fig. 5F); the displacement due to simulations of expansion angle (Fig. 5F) and

**Table 1** Sources of variation in sexual ornamentation of house finches

Factor	Source							
	Population		Age		Ornamental part		Population $\times$ Age	
	F	P	F	P	F	P	F	P
Overall ornament area	3.99	0.05	0.90	0.34	269.8	<0.01	1.54	0.14
Overall ornament hue	0.30	0.59	2.02	0.09	5.02	<b>0.01</b>	5.33	<0.01
Feather size	5.38	0.02	0.38	0.54	2485.7	<0.01	3.03	0.08
% Feather pigmented	14.37	<0.01	7.02	<0.01	331.4	<0.01	0.14	0.71
Feather barb length	1.16	0.28	0.13	0.72	315.2	<0.01	0.17	0.68
Feather isochronic angle	3.60	0.06	8.48	<0.01	11.47	<b>0.04</b>	0.21	0.65

F-values are from ANOVA.

Bold values indicate significance at  $\alpha = 0.05$ .

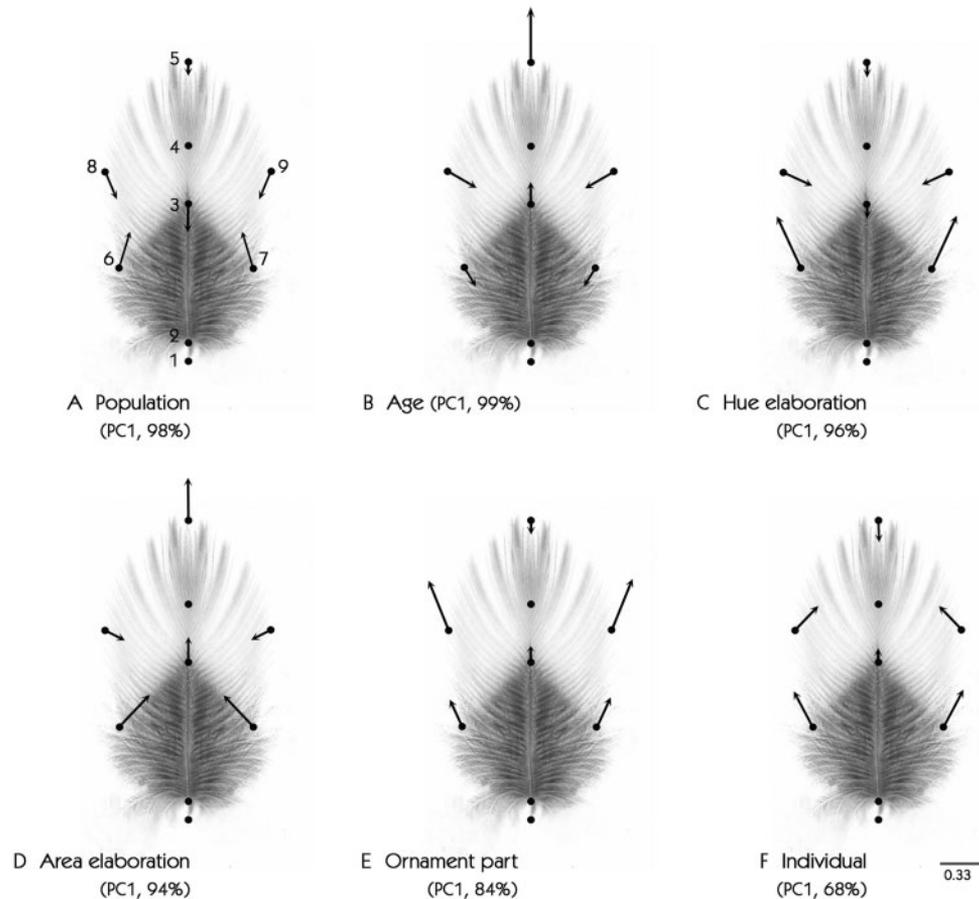


**Fig. 2** Descriptive statistics of sexual ornamentation in house finches for each population, age, and ornamented part. Shown are mean  $\pm$  1 SE of ornament's (A) area, (B) hue, (C) size of ornamented feather, (D) proportion of ornamented feather that is pigmented, (E) length of uppermost barb, and (F) isochronic angle (angle formed by landmarks 6-3-7). Asterisks indicate significant differences between ages within each group. Table 1 shows other tests.

**Table 2** Variance components (% variance) for displacement of ornamental landmarks (left side of feather only) due to the effects in the Procrustes ANOVA of feather shape

Landmarks	Source						
	Population	Age	Hue	Area	Part	Individual	
3	<u>14.4</u>	<u>29.3</u>	3.6	<u>44.7</u>	6.2	1.8	
5	4.4	<u>41.6</u>	1.5	<u>38.5</u>	1.5	12.5	
6	<u>14.7</u>	6.8	<u>31.8</u>	<u>16.9</u>	11.8	<u>18.0</u>	
8	11.4	12.2	13.3	5.1	39.3	<u>18.7</u>	

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



**Fig. 3** Observed landmark displacement of fully-grown feathers due to (A) population affiliation, (B) age (1-year old versus 2-year old), (C) elaboration of overall hue, (D) overall ornamented area, (E) ornamented body part, (F) among-individual variation. PC coefficients are shown as vectors originating at the mean configuration for each landmark and the 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. The length of isometric loading (0.33 with  $n=9$  landmarks) is shown for scale. All landmarks were used in calculations, but only the displacements of landmarks delineating ornamental part of the feather are shown.

initial number of barbs was similar ( $r_s=0.71$ ,  $\gamma=44.6^\circ$ , Fig. 5C).

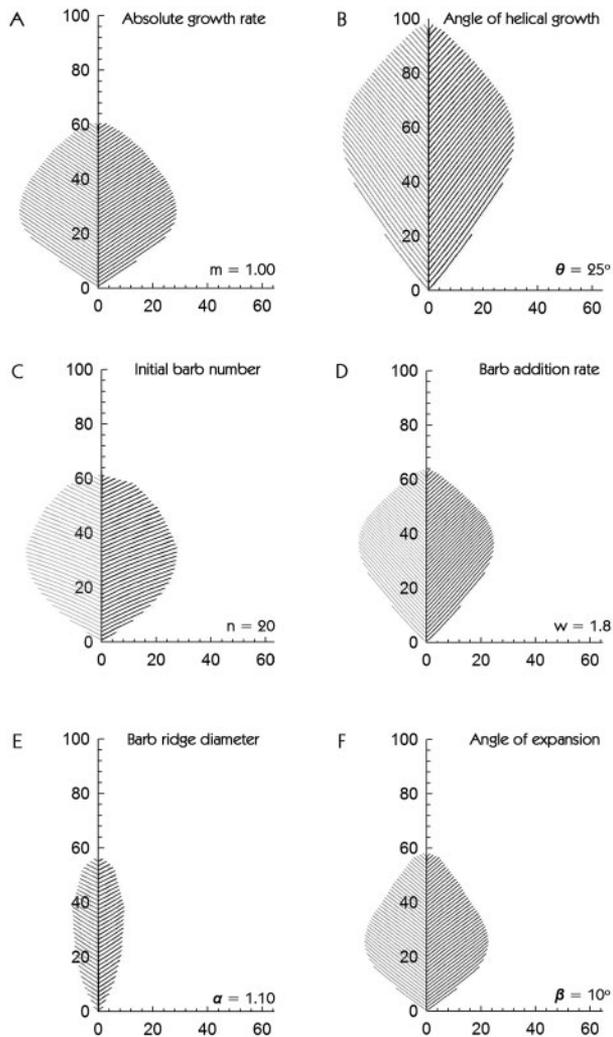
### Concordance of observed and predicted patterns of displacement

Simulated patterns of landmark displacement due to absolute growth rate (Fig. 5A) were statistically indistinguishable from observed among-individual variation (Figs. 3F and 6F), patterns simulated with variation of helical angle of growth (Fig. 5B) were identical to observed differences between males ages (Figs. 3B and 6B), whereas patterns simulated with variation in initial number of barbs (Fig. 5C) were concordant with observed differences in feather shapes among ornamental parts (Figs. 3E and 6E). On the other hand, observed variation due to overall hue elaboration and ornamental body part were concordant with displacement caused by all but one simulated parameter (Fig. 6C, hue elaboration: all parameters except for simulated barb diameter;

Fig. 6E, ornamental part: all parameters except for simulated barb addition rate). Simulated variation in rate of addition of barbs and in helical angle of growth were indistinguishable from observed variation in both between-population variation (Fig. 6A) and, along with simulated variation in barb diameter, with overall ornamental area (Fig. 6D).

### Discussion

Our study of the developmental basis of pigmentation of ornamental feathers has produced four main results. First, we found close concordance between individual variation in feather shape and hue elaboration, as well as close concordance between age-related changes in ornamentation and patterns of feather growth. These findings uncover, for the first time, proximate mechanisms for regulation of variation in sexual ornamentation in relation to



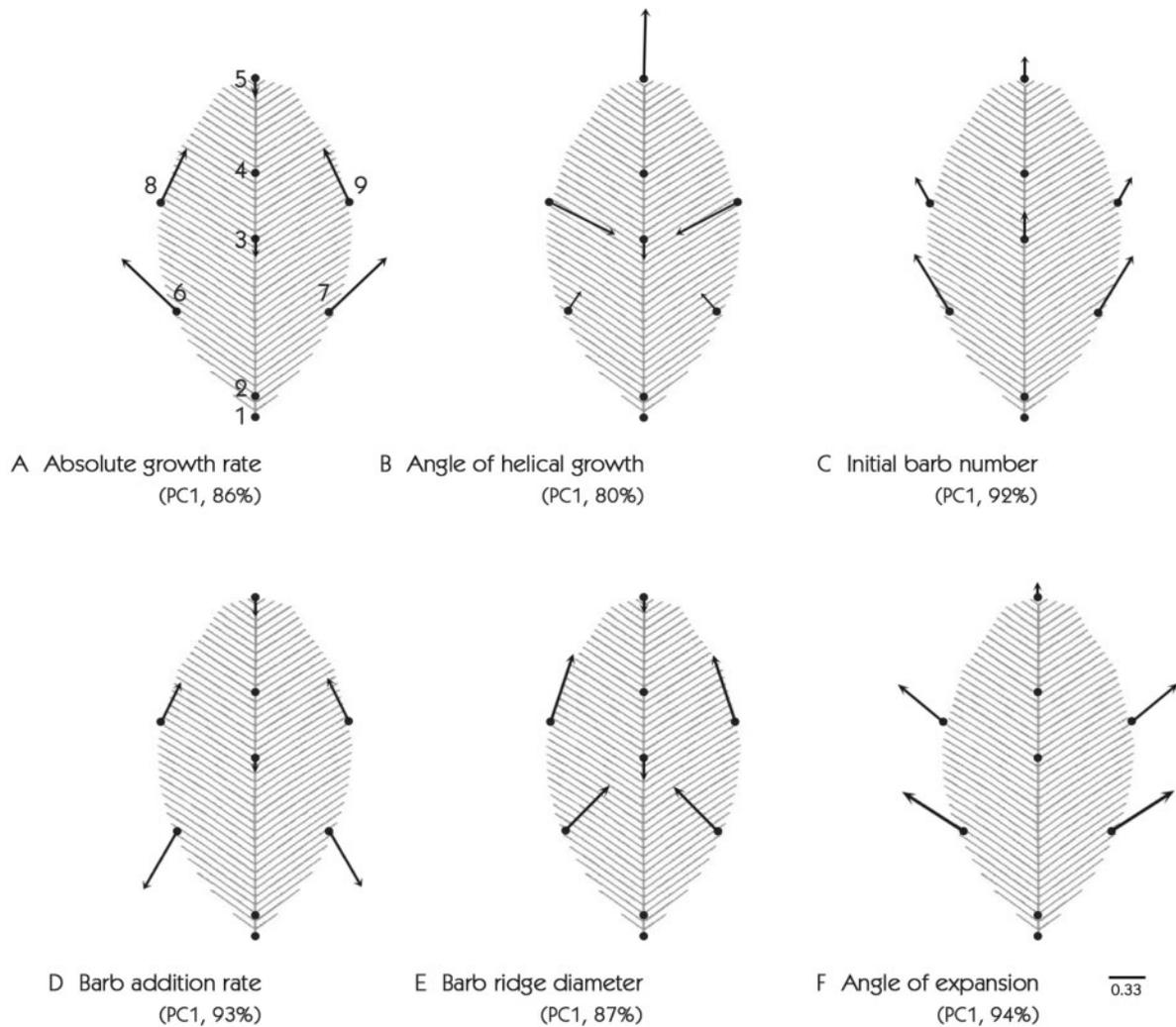
**Fig. 4** Ornamental feathers simulated by varying (A) absolute growth rate, (B) angle of helical growth, (C) initial barb number, (D) barb addition rate, (E) barb ridge diameter, (F) angle of ramus expansion, while keeping all other parameters constant. Shown are parameter values and corresponding feather shapes at the end of simulated growth.

age, context and condition in a model species for studies of sexual selection on plumage ornamentation (Hill et al. 1999; Badyaev and Duckworth 2003; Hill 2003; Badyaev and Young 2004). Second, we found significant concordance between within-population patterns of ornament elaboration and patterns of population divergence, suggesting the link between the development and evolution of diversification in shape of feathers. Third, the simulated patterns produced by modeling feather growth adequately captured observed variation in ornamental shape variation, corroborating previous suggestions of the defining role of within-follicle feather growth in feather pigmentation (Lillie and Juhn 1932; Willier 1941; Nickerson 1944; Brumbaugh 1967; Brush and

Seifried 1968; Prum and Williamson 2002). Finally, we found significant variation in developmental determination of feather shape; comparison of simulated and observed growth of feathers revealed that a single simulated parameter of growth adequately accounts for age-related differences in pigmentation, whereas variation in the shape of feathers due to hue, ornament area, and ornamental body part covaried with several equally important parameters of growth (Fig. 6). We now will address these four main results in turn.

The finding of close integration of the growth and pigmentation of feathers has several important implications. First, it provides a developmental mechanism for the evolution of condition-dependence in sexual ornamentation—a topic of great interest in studies of sexual selection (Andersson and Simmons 2006). Second, the complexity, redundancy, and environmental lability, typical of developmental integration of growth and pigmentation of feathers, should facilitate developmental retention and accommodation of novel carotenoid pigments into feather ornamentation and, thus, could be an important source of evolutionary diversification in carotenoid-based displays (Badyaev 2006, 2007). Finally, large influence of several components on feather growth might limit patterns of evolutionary diversification in the elaboration of plumage ornamentation, accounting for commonly documented convergence in color patterns across distinct lineages.

Our results show that diversification in shapes of ornamental feathers can be understood in terms of processes operating at the level of the follicle. For example, pronounced variation in shape of ornamental feathers across ornamental parts (Fig. 1B–D) was adequately explained by variation in number, diameter, and absolute growth rate of barbs (Fig. 6E), corroborating empirical observations that follicle diameter varies across feather tracks (Prum and Williamson 2001; Harris et al. 2005). However, what integrates feather growth with carotenoid-based pigmentation proximately is still an open question. It is thought that the exceptional ability of the follicle to accommodate diverse and novel environmental and epigenetic inputs during development (Yue et al. 2005; Alibardi and Sawyer 2006; Lin et al. 2006; Yue et al. 2006) should make follicular processes susceptible to hormonal regulation that accomplishes not only the production of age-specific and sex-specific shapes and colorations of feathers (Widelitz et al. 2003; Jiang et al. 2004; Arevalo and Heeb 2005; Lin et al. 2006), but also forms precise color patterns that require close integration among many follicles (Lucas and Stettenheim 1972; Prum and Dyck 2003).

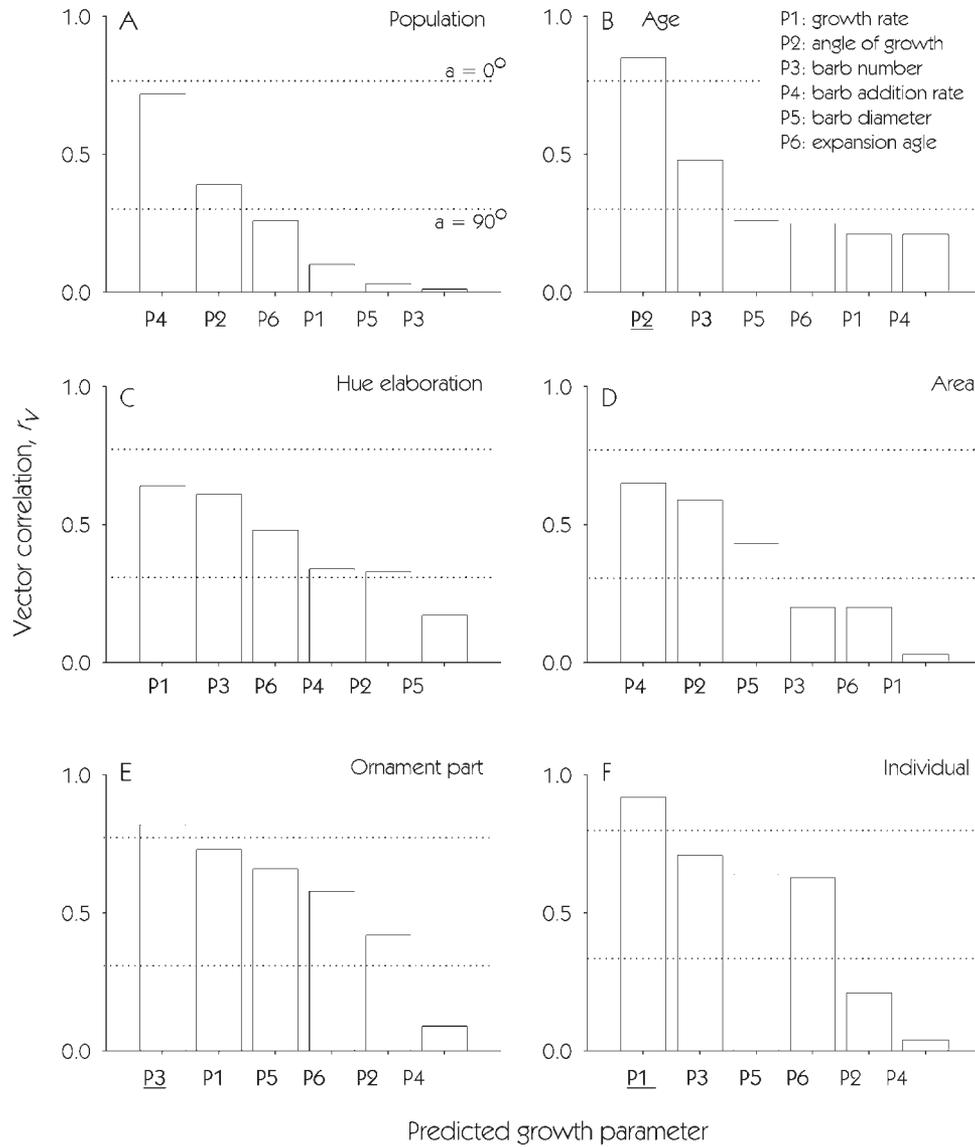


**Fig. 5** Predicted landmark displacement of feathers simulated by varying (A) absolute growth rate, (B) angle of helical growth, (C) initial barb number, (D) barb addition rate, (E) barb ridge diameter, (F) angle of ramus expansion. PC coefficients are shown as vectors originating at the mean configuration for each landmark for each growth component and the length and direction of PC coefficients. Numbers are percent of variation accounted by PC1 for each growth component. The length of isometric loading (0.33 with  $n=9$  landmarks) is shown for scale. All landmarks were used in calculations, but only the displacements of the landmarks delineating the ornamental part of the feather are shown.

Such epigenetic regulation of feather coloration can harbor considerable condition-dependence (see subsequently). However, when close and consistent developmental integration between growth and pigmentation are favored by selection for a particular color pattern, it can lead to the evolution of genetic correlations between growth and pigmentation components (Badyaev 2004; Young and Badyaev 2006), as is evident in genetics of feather pigmentation in some poultry lineages (Somes 1980, 2003; Minvielle et al. 2005).

Close developmental integration of growth and pigmentation of feathers, and their potentially shared hormonal regulation, offers a multitude of targets for

selection for elaboration of the plumage. In the house finch, the shared effect of prolactin on both parental care and on molt of sexual ornamentation enables parental males to develop equally elaborated sexual ornamentation compared to nonparental males, despite being in lower physiological condition at the time of molt (Duckworth et al. 2003; Badyaev and Vleck 2007). This study suggests that variation in rate of feather growth, known to be regulated by prolactin (Dawson 2006), could provide a proximate mechanism by which prolactin's effect on feather growth can be translated into elaboration of sexual ornamentation. Similarly, greater ornamentation of older males (Fig. 2D) and apparently faster growth of



**Fig. 6** Concordance between predicted and observed ornamental-landmark displacement for variation between (A) populations, (B) ages, (C) hue elaboration, (D) ornament areas, (E) ornament parts, and (F) individuals. Shown are vector correlations between PC1 of Procrustes mean squared of observed sample and PC1 of predicted sample of the simulated parameters: P1—absolute growth rate, P2—angle of helical growth, P3—initial number of barbs, P4—barb rate of addition of barbs, P5—barb ridge diameter, and P6—angle of ramus expansion. Correlations above upper dashed line (underlined predictions) indicate the angles not different from  $0^\circ$  (i.e., complete concordance), correlations between the two dashed lines (predictions shown in bold) indicate angles significantly different from both  $0^\circ$  and  $90^\circ$ , correlations below lower dashed line indicate angles not different from  $90^\circ$  (i.e., complete discordance)

their ornamental feathers (smaller isochronic angle, Figs. 2F, 4B), is corroborated by the finding that a single developmental parameter—the angle of helical growth—can account for age differences in feather ornamentation (Figs. 5B and 6B).

Developmental integration of the growth and ornamentation of feathers can facilitate the evolution of condition-dependence in sexual ornamentation. Feather growth and associated keratin metabolism account for 20–40% of the expenditure of dry body weight during the molt period (Dawson et al. 2000).

Moreover, close coordination of feather growth across ornamental parts and dependence of pigmentation patterns on the organism-wide transportation and metabolism of carotenoid precursors further enhance condition-dependence of sexual ornamentation (Dawson 2003; Badyaev 2007). Our finding of close concordance between among-individual variation and variation in hue elaboration (Fig. 3C and F) not only suggests that a common mechanism—absolute growth rate (Fig. 6C and F)—underlies both of these patterns, but, more importantly,

also explicitly attributes variation in sexual ornamentation to differences among individuals. Thus, selection on hue elaboration in this species could act proximately on individual differences in growth rate of feathers.

A finding that only six components of growth can produce significant diversification in feather shapes suggests that the pathways for such diversification are limited, resulting in frequently observed parallel and convergent evolution of feather displays (Lucas and Stettenheim 1972; Prum and Williamson 2002). At the same time, complexity and redundancy of association among the components of feather growth could produce compensatory interactions among them (Young and Badyaev 2007) and thus account for the continuous variation in color ornamentation found in some lineages (Omland and Hofmann 2005). In addition, such redundancy and environmental lability should enable developmental retention and incorporation of novel pigments, and, thus, facilitate genetic assimilation of novel carotenoid compounds (Badyaev 2007). This idea is corroborated by frequent finding of modified feather structure following deposition of carotenoid pigments, including novel compounds (Troy and Brush 1983; Hudon and Brush 1989; Hudon 1991; Bleiweiss 2004).

In summary, we show that variation in the parameters of feather growth can account for significant diversification in carotenoid-based ornamentation. Our study reveals proximate targets of selection for the elaboration of sexual ornamentation and, in combination with detailed examination of pigment metabolism (Brush 1978, 1990; McGraw 2006), provides a testable framework for empirical investigations of the functional and evolutionary diversification of avian color displays.

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## References

- Alibardi L, Sawyer RH. 2006. Cell structure of developing downfeathers in the zebrafinch with emphasis on barb ridge morphogenesis. *J Anat* 208:621–42.
- Andersson M, Simmons LW. 2006. Sexual selection and mate choice. *Trends Ecol Evol* 21:296–302.
- Arevalo JE, Heeb P. 2005. Ontogeny of sexual dimorphism in the long-tailed Manakin (*Chiroxiphia linearis*): long maturation of display trait morphology. *Ibis* 147:697–705.
- Badyaev AV. 2004. Developmental perspective on the evolution of sexual displays. *Evol Ecol Res* 6:975–91.
- Badyaev AV. 2006. Colorful phenotypes of colorless genotypes: towards a new evolutionary synthesis of animal color displays. In: Hill GE, McGraw KJ, editors. *Bird coloration: function and evolution*. Cambridge: Harvard University Press. p 349–379.
- Badyaev AV. 2007. Evolvability and robustness in color displays: bridging the gap between theory and data. *Evol Biol*. In press.
- Badyaev AV, Duckworth RA. 2003. Context-dependent sexual advertisement: plasticity in development of sexual ornamentation throughout the lifetime of a passerine bird. *J Evol Biol* 16:1065–76.
- Badyaev AV, Foresman KR. 2000. Extreme environmental change and evolution: stress-induced morphological variation is strongly concordant with patterns of evolutionary divergence in shrew mandibles. *Roy Soc Lond* 267:371–7.
- Badyaev AV, Hill GE. 2003. Avian sexual dichromatism in relation to ecology and phylogeny. *Annu Rev Ecol Syst* 34:27–49.
- Badyaev AV, Hill GE, Dunn PO, Glen JC. 2001. Plumage color as a composite trait: developmental and functional integration of sexual ornamentation. *Am Nat* 158:221–35.
- 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*. In press.
- Badyaev AV, Young RL. 2004. Complexity and integration in sexual ornamentation: an example with carotenoid and melanin plumage pigmentation. *J Evol Biol* 17:1317–27.
- Baker RR, Parker GA. 1979. The evolution of bird coloration. *Philos T Roy Soc* 287:65–130.
- Bartels T. 2003. Variations in the morphology, distribution, and arrangement of feathers in domesticated birds. *J Exp Zool* 298B:91–108.
- Bleiweiss R. 2004. Novel chromatic and structural biomarkers of diet in carotenoid-bearing plumage. *P Roy Soc Lond* 271:2327–35.
- Bortolotti GR, Blas J, Negro JJ, Tella JL. 2006. A complex plumage pattern as an honest social signal. *Anim Behav* 72:423–430.
- Britton G. 1998. Overview of carotenoid biosynthesis. In: Britton G, Pfander H, Liaaen-Jensen S, editors. *Carotenoids: biosynthesis and metabolism*. Basel: Birkhäuser. p 13–147.

- Brumbaugh JA. 1967. Differentiation of black-red melanin in the fowl: interaction of pattern genes and feather follicle milieu. *J Exp Zool* 166:11–24.
- Brush AH. 1978. Avian pigmentation. In: Brush AH, editor. *Chemical zoology*. New York: Academic Press. p 141–61.
- Brush AH. 1990. Metabolism of carotenoid pigments in birds. *FASEB* 4:2969–77.
- Brush AH, Seifried H. 1968. Pigmentation and feather structure in genetic variants of the Gouldian finch, *Poephila gouldiae*. *Auk* 85:416–30.
- Burt E. 1986. An analysis of physical, physiological, and optical aspects of avian coloration with emphasis on wood-warblers. *Ornithol Monogr* 38:1–122.
- Cheng KM, Brush AH. 1984. Feather morphology of four different mutations in the Japanese quail. *Poult Sci* 63:391–400.
- Chetverikov SS. 1926. On certain aspects of the evolutionary process from the standpoint of modern genetics. *J Exp Biol A* 2:1–40.
- Chuong C, Chodankar R, Wideltz RB, Jiang T. 2000. Evo-devo of feathers and scales: building complex epithelial appendages. *Curr Op Gen Dev* 10:449–56.
- Dawson A. 2003. A detailed analysis of primary feather moult in the Common starling *Sturnus vulgaris*—new feather mass increases at a constant rate. *Ibis* 145:69–76.
- Dawson A. 2006. Control of molt in birds: association with prolactin and gonadal regression in starlings. *Gen Comp Endocrin* 147:314–22.
- Dawson A, Hinsley SA, Ferns PN, Bonser RHC, Eccleston L. 2000. Rate of moult affects feather quality: a mechanism linking current reproductive effort to future survival. *Roy Soc Lond* 267:2093–8.
- Duckworth RA, Badyaev A, Parlow AF. 2003. Males with more elaborated sexual ornaments avoid costly parental care in a passerine bird. *Behav Ecol Sociobiol* 55:176–83.
- Eames BF, Schneider RA. 2005. Quail-duck chimeras reveal spatiotemporal plasticity in molecular and histogenic programs of cranial feather development. *Development* 132:1499–509.
- Fox DL. 1976. *Avian biochromes and structural colors*. Berkeley: University of California Press.
- Goodall C. 1991. Procrustes methods in the statistical analysis of shape. *JR Stat Soc B* 53:285–339.
- Harris MP, Fallon JF, Prum RO. 2002. Shh-Bmp2 signaling module and the evolutionary origin and diversification of feathers. *J Exp Zoology (Mol Dev Evol)* 294:160–76.
- Harris MP, Williamson S, Fallon JF, Meinhardt H, Prum RO. 2005. Molecular evidence for an activator-inhibitor mechanism in development of embryonic feather branching. *PNAS* 102:11734–9.
- Hill GE. 2003. *A red bird in a brown bag: the function and evolution of colorful plumage in the house finch*. New York: Oxford Ornithology Series.
- Hill GE, McGraw KJ, editors. 2006. *Bird coloration: mechanisms and measurements*. Cambridge, MA: Harvard University Press.
- Hill GE, Nolan PM, Stroehr AM. 1999. Pairing success relative to male plumage redness and pigment symmetry in the house finch: temporal and geographic constancy. *Behav Ecol* 10:48–53.
- Hudon J. 1991. Unusual carotenoid use by western tanager (*Piranga ludoviciana*) and its evolutionary implications. *Can J Zool* 69:2311–20.
- Hudon J, Brush AH. 1989. Probably dietary basis of a color variant of the cedar waxwing. *J Field Ornithol* 60:361–8.
- Huxley JS. 1942. *Evolution, the Modern Synthesis*. Harper and Brothers, New York.
- Jiang TX, Wideltz RB, Shen WM, Will P, Wu DY, Lin CM, Jung HS, Chuong CM. 2004. Integument pattern formation involves genetic and epigenetic controls: feather arrays simulated by digital hormone models. *Int J Dev Biol* 48:117–35.
- Kim JY, et al. 2005. Formation of spacing pattern and morphogenesis of chick feather buds is regulated by cytoskeletal structures. *Differentiation* 73:240–8.
- Klingenberg CP, McIntyre GS. 1998. Geometric morphometrics of developmental instability: analyzing patterns of fluctuating asymmetry with Procrustes methods. *Evolution* 53:1363–75.
- Lewontin RC. 1983. Gene, organism and environment. In: Bendall DS, editor. *Evolution: from molecules to men*. Cambridge: Cambridge University Press. p 273–85.
- Lillie FR, Juhn M. 1932. The physiology of development of feathers. I. Growth-rate and pattern in individual feathers. *Phys Zoöl* 5:124–84.
- Lin CM, Jiang TX, Wideltz RB, Chuong CM. 2006. Molecular signaling in feather morphogenesis. *Curr Opin Cell Biol* 18:730–41.
- Lucas AM, Stettenheim PR. 1972. *Avian anatomy: integument*. USDA, Washington, DC.
- Mayr E. 1942. *Systematics and the origin of species*. New York: Dover.
- McGraw KJ. 2006. The mechanics of carotenoid coloration in birds. In: Hill GE, McGraw KJ, editors. *Bird coloration. I. Mechanisms and measurements*. Cambridge, MA: Harvard University Press. p 177–242.
- Minvielle F, Gourichon D, Moussu C. 2005. Two new plumage mutations in the Japanese quail: “curly” feather and “rusty” plumage. *BMC Genet* 6:1–5.
- Muller GB, Newman S. 2003. *Origination of organismal form: beyond the gene in developmental and evolutionary biology*. Cambridge: The MIT Press.
- Nickerson M. 1944. An experimental analysis of barred pattern formation in feathers. *J Exp Zool* 95:361–94.
- Omland KE, Hofmann CM. 2005. Adding color to the past: ancestral-state reconstruction of coloration. In: Hill GE, McGraw KJ, editors. *Bird coloration: function and evolution*. Cambridge: Harvard University Press. p 417–54.
- Orr HA. 2005. The genetic theory of adaptation: a brief history. *Nat Rev Genet* 6:119–27.
- Oyama S, Griffiths PE, Gray RD. 2001. *Cycles of contingency: developmental systems and evolution*. Cambridge: MIT Press.

- Price T, Chi E, Pavelka M, Hack M. 1991. Population and developmental variation in the feather tip. *Evolution* 45:518–33.
- Prum RO, Brush AH. 2002. The evolutionary origin and diversification of feathers. *Quart Rev Biol* 77:261–95.
- Prum RO, Dyck J. 2003. A hierarchical model of plumage: morphology, development, and evolution. *J Exp Zool* 298B:73–90.
- Prum RO, Williamson S. 2001. Theory of the growth and evolution of feather shape. *J Exp Zool (Mol Dev Evol)* 291:30–57.
- Prum RO, Williamson S. 2002. Reaction-diffusion models of within-feather pigmentation patterning. *Proc Roy Soc Lond* 269:781–92.
- Rohlf FJ, Slice D. 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. *Syst Zool* 39:40–59.
- Rouzankina I, Abate-Shen C, Niswander L. 2004. Dlx genes integrate positive and negative signals during feather bud development. *Dev Biol* 265:219–33.
- Somes RGJ. 1980. The mottling gene, the basis of six plumage color patterns in the domestic fowl. *Poult Sci* 59:1370–4.
- Somes RGJ. 2003. Mutations and major variants of plumage and skin in chickens. In: Crawford RD, editor. *Poultry breeding and genetics*. Amsterdam: Elsevier. p 169–208.
- Troy DM, Brush AH. 1983. Pigments and feather structure of the redpolls, *Carduelis flammea* and *C. hornemanni*. *Condor* 85:443–6.
- Voitkevich AA. 1966. *The feathers and plumage of birds*. New York: October House, Inc.
- West-Eberhard MJ. 2003. *Developmental plasticity and evolution*. Oxford: Oxford University Press. p 795.
- Widelitz RB, Jiang TX, Yu M, Shen T, Shen J-Y, Wu P, Yu Z, Chuong CM. 2003. Molecular biology of feather morphogenesis: a testable model for evo-devo research. *J Exp Zool* 298B:109–22.
- Willier BH. 1941. An analysis of feather color pattern produced by grafting melanophores during embryonic development. *Am Nat* 75:136–46.
- Young RL, Badyaev AV. 2006. Evolutionary persistence of phenotypic integration: influence of developmental and functional relationships on evolution of complex trait. *Evolution* 60:1291–9.
- Young RL, Badyaev AV. 2007. Evolution of ontogeny: linking epigenetic remodeling and genetic adaptation in skeletal structures. *Integr Comp Biol*. 10.1093/icb/icm025.
- Yu MK, *et al.* 2004. The developmental biology of feather follicles. *Int J Dev Biol* 48:181–91.
- Yue Z, Jiang T-X, Widelitz RB, Chuong C-M. 2005. Mapping stem cell activities in the feather follicle. *Nature* 438:1026–9.
- Yue ZC, Jiang TX, Widelitz RB, Chuong CM. 2006. Wnt3a gradient converts radial to bilateral feather symmetry via topological arrangement of epithelia. *PNAS* 103:951–5.