Evolution of the Morphological Innovations of Feathers

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ABSTRACT   Feathers are complex assemblages of multiple morphological innovations. Recent research on the development and evolution of feathers has produced new insights into the origin and diversification of the morphological innovations in feathers. In this article, I review and discuss the contribution of three different factors to the evolution of morphological innovations in feathers: feather tubularity, hierarchical morphological modularity, and the co-option molecular signaling modules. The developing feather germ is a tube of epidermis with a central dermal pulp. The tubular organization of the feather germ and follicle produces multiple axes over which morphological differentiation can be organized. Feather complexity is organized into a hierarchy of morphological modules. These morphological modules evolved through the innovative differentiation along multiple different morphological axes created by the tubular feather germ. Concurrently, many of the morphological innovations of feathers evolved through the evolutionary co-option of plesiomorphic molecular signaling modules. Gene co-option also reveals a role for contingency in the evolution of hierarchical morphological innovations. *J. Exp. Zool. (Mol. Dev. Evol.) 304B:570–579, 2005.*   © 2005 Wiley-Liss, Inc.

Feathers are an outstanding example of a hierarchically complex morphological innovation (Prum, '99; Prum and Dyck, 2003). As the other articles in this symposium emphasize, the origin of morphological innovations, or evolutionary novelties, provides special challenges to the field of evolutionary biology. The study of the evolution of morphological innovations has particularly benefited from the application of concepts and data from developmental evolutionary biology. Here, I will review a few select topics on the developmental evolutionary biology of feathers. In particular, I will ask, "What aspects of the developmental biology of feathers have contributed to the evolution of their morphological innovations?"

Feathers are characterized by a *complex* of multiple morphological and biochemical innovations. The most obvious morphological innovation in feathers is their complex branching structure of the rachis, barbs, and barbules (Lucas and Stettenheim, '72; Prum, '99). The incredible array of morphological variations of this basic structure has given rise to an amazing diversity of structures. This explosive diversity in feather structure is exploited for a wide variety of functions in the lives of birds, and their theropod ancestors (Prum and Brush, 2002), including flight, insulation, visual communication, crypsis, and even sound production and water transport (Stettenheim, '76). Feathers are fundamental to the biology of living birds, and their diversity of structure and versatility in function have certainly contributed to the status of birds as the most diverse clade of terrestrial vertebrates.

After a century of scientific literature regarding feathers as specialized scales (reviewed in Lucas and Stettenheim, '72), Brush ('93, '96) was the first to recognize and emphasize the overwhelming novelty of feathers. Although Brush focused most on the novelties of the biochemistry feather keratins from other reptilian beta-keratins, he recognized that the key to understanding the evolutionary origin of feathers was to be found in

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unraveling the origin of feather *innovations*, not to reconstruct a transition series of plesiomorphic similarities shared by feathers and archosaurian scales.

Prum ('99) and Brush (2000, 2001) then focused specifically on *developmental* approaches to understanding the origin of the morphological innovations of feathers. Specifically, Prum ('99) proposed an explicitly developmental theory of the origin and evolution of feathers. I hypothesized that feather diversity evolved through a series of evolutionary novelties in the developmental mechanisms of the feather follicle and feather germ (Prum, '99). The model hypothesized a hierarchical series of stages in feather evolution, each of which is characterized by an additional novelty in developmental mechanisms. The theory also predicted a transition series in the evolution of the morphological innovations of feathers. In brief, these stages are characterized by the successive evolution of tubularity, barbs, the rachis and barbules, and, finally, the closed pennaceous vane. Subsequently, some of the major morphological predictions of the theory—such as the prediction that filamentous feathers evolved in theropod dinosaurs before the pennaceous planar vane—have been supported by new paleontological discoveries from Liaoning, China (Sues, 2001; Norell et al., 2002; Prum and Brush, 2002).

Recent molecular work by Harris et al. (2002) and Yu et al. (2002) has begun to document the molecular mechanisms of the development of the morphological innovations of feathers. In particular, Harris et al. (2002) provide molecular details of the co-option of molecular signaling modules in the evolution of feather complexity.

In this review, I will focus on describing the contributions of three different factors to the macroevolution involved in the origin of morphological innovations in feathers: (1) the tubular organization of the feather germ, (2) the hierarchical modularity of feather innovations, and (3) the evolutionary co-option of genetic signaling modules in the development of the morphological innovations of feathers.

FEATHERS ARE TOTALLY TUBULAR

Traditionally, feathers have included a broad diversity of integumentary appendages of birds excluding their scales. However, the developmental model of the evolution of feathers provided the first explicit definition of feathers: feathers are hollow tubes of keratinocytes that grow from a feather follicle (Prum, '99). The feather follicle is a cylindrical epidermal invagination that develops around the first tubular outgrowth or short bud. Subsequently, Harris et al. (2002) emphasized that the evolutionary origin of the tubular feather germ itself marked the origin of the feather. Although all extant feathers grow from a tubular feather follicle, Harris et al. (2002) decoupled the origin of the first tubular feather germ from the follicle itself. Although all extant feathers grow from follicles, it is possible that the innovation of an initially tubular appendage evolved before the origin of the feather follicle. However, it is unlikely that this structure could have been renewable or regenerated through molt without a true, tubular follicle at its base.

The fundamental tubularity of all feathers is clearly demonstrated by their form and growth. All feathers grow from a tubular feather germ of epidermis with dermal pulp tissue at the center (Fig. 1). The extent of the dermal pulp within the feather germ is limited by a series of pulp caps that cover the distal surface of the pulp (Fig. 1). As the feather grows out of the skin by the proliferation of new epidermal cells at the base of the feather germ, a series of new pulp caps grows, and the dermal pulp within the most distal pulp cavity is reabsorbed (Lillie, '40). When the feather germ is mature, the superficial sheath falls off, the differentiated barbs expand, and the interior surface of the tube becomes the reverse or bottom surface of the planar vane (Lucas and Stettenheim, '72; Prum, '99; Prum and Williamson, 2001). Pulp caps are produced throughout the growth of the feather vane (after about day 15 in a regenerating chicken feather) (Lillie, '40). When the feather vane unfurls, the fine pulp caps within this portion of the germ fall apart and are lost, but the pulp caps remain within the calamus, which is the tubular structure at the base of all feathers that does not unfurl.

The tubular nature of feathers is further demonstrated by the continuity of the tubular organization through the multiple feathers that grow in series out of a single feather follicle during the life of a bird through molt. At the distal end of the calamus, the central dermal cavity that was opened with the unfurling of the feather vane passes into the center of the keratinized tubular calamus through a hole called the *superior umbilicus* (Lucas and Stettenheim, '72, Figs. 158–159). At the base of the calamus, another hole, called the *inferior umbilicus*, passes out from the central cavity occupied by the dermal pulp
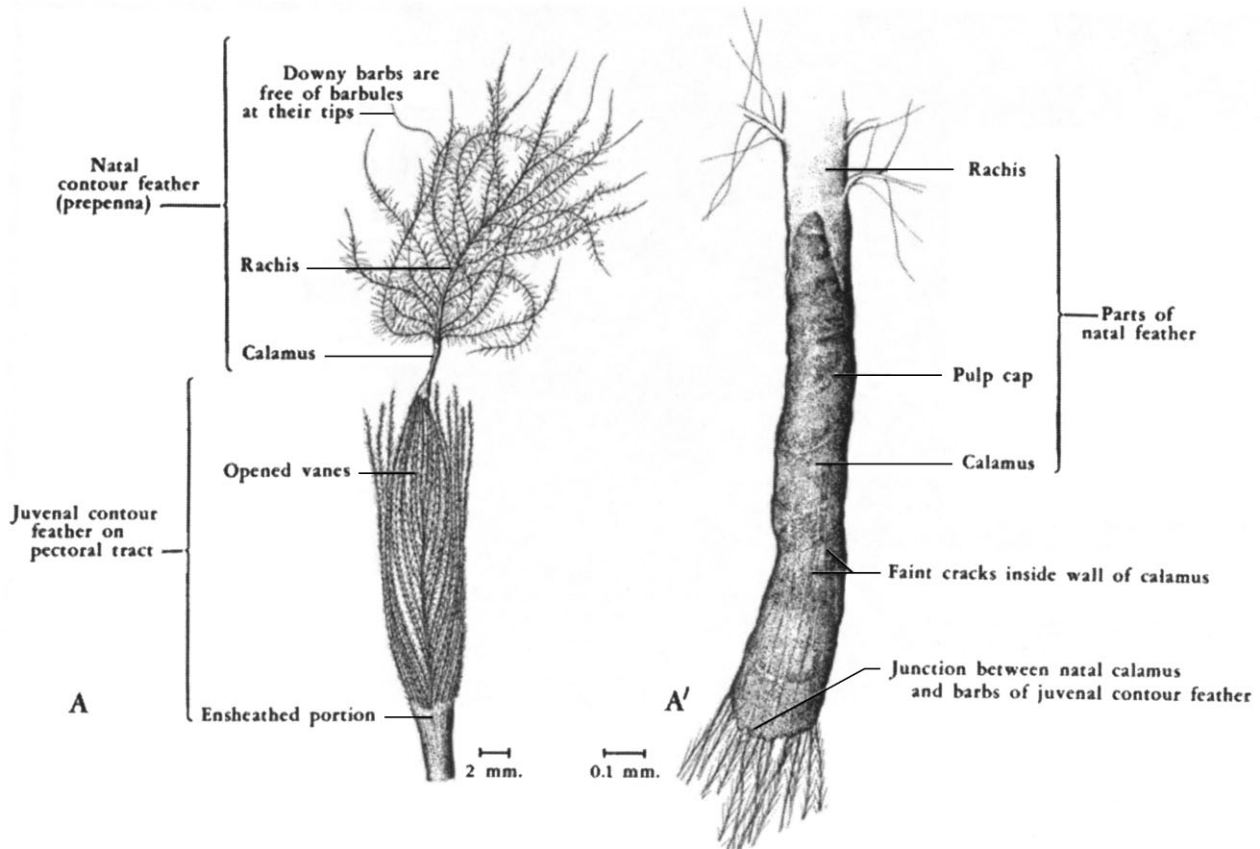
Fig. 1.   Illustration of the contiguity of the tubular epidermis and central dermal pulp between generations of feathers that grow from the same follicle shown by the natal down and juvenal contour feathers of a White Pekin Duck, from Lucas and Stettenheim ('72, Fig. 229). (**A**) The natal feather has been replaced by the new juvenal feather growing out of the same follicle. (**A'**) The distal tips of the new juvenal feather are connected to the base of the tubular calamus of the older natal feather. The opening into the dermal pulp cavity of the natal feather (called the inferior umbilicus) is contiguous with the central dermal pulp lumen of the juvenal feather germ.

(Lucas and Stettenheim, '72, Figs. 158–159). When the molt of the first natal down begins after hatching, the tips of the first barbs of the juvenal feathers are often physically connected to the base of the calamus of the previous natal down feather to have grown from that follicle (Fig. 2).

The inferior umbilicus of a feather is completely coherent and continuous with the distal tip of the subsequent feather to grow from that follicle (Fig. 2). The very first embryonic feather germ to develop in the egg are each initially cones of epidermis with a central dermal pulp (Lucas and Stettenheim, '72; Prum, '99; Harris et al., 2002). But once the follicle is developed with the follicular collar restricting the dermal pulp to a central lumen within the feather germ, the tube of epidermis that forms the feathers themselves and the lumen occupied by the dermal pulp at the center of the tubular feather germ is physically
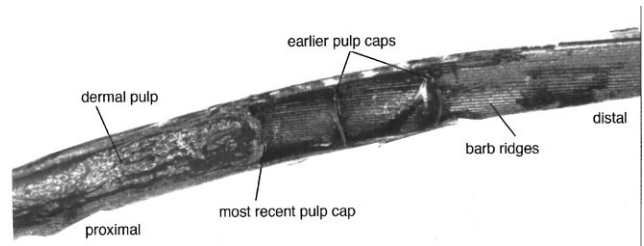

Fig. 2.   Photograph of a longitudinal (anterior–posterior) section of the developing tubular germ from the pennaceous contour feather of a blue-and-yellow Macaw (*Ara ararauna*, Psittaciformes) (proximal left, distal right). The section shows the central dermal pulp (left) at the base of the feather germ that is limited by the most recent pulp cap. In the center of the section are two earlier pulp caps. The spaces between pulp caps show the compartment of dermal pulp that is reabsorbed during the production of the next pulp cap. The presence of helically growing barb ridges shows that pulp caps are produced throughout the growth of the tubular feather germ, not just during the development of the calamus.

continuous between all of the feathers that grow from that follicle. This interconnection exists between all subsequent feathers grown from the follicle, but is not easily observed because larger feathers usually fall out of the follicle as the new feather begins to grow. Thus, the feathers grown from a single follicle during the life of a bird are all iterated derivations, or homologous serial sections, of the same continuous tube (Prum and Dyck, 2003).

The continuity of the tubular epidermis and the central dermal pulp among feather generations can also be seen in unusual tubular feathers which entirely lack a vane. For example, cassowaries (*Casuarius*) are flightless ratites with highly simplified wing feathers, or remiges, that are essentially 15–25 cm long, hollow structures with a series of pulp caps dividing the central passage. Because an entire cassowary wing feather is essentially an undifferentiated, emergent calamus, the distal and basal tips of a cassowary wing feather show the superior umbilicus and inferior umbilicus, respectively, which can be easily seen at the ends of any cassowary wing feather. Cassowary wing feathers are replaced periodically during molt, further demonstrating the tubular continuity of all the feathers grown from a follicle. Other, entirely tubular feathers can be found on the crown of the Horned Screamer (*Anhima cornuta*, Anhimidae) and the crown of some male African Peacock (*Afropavo congensis*, Phasianidae). These unusual, derived feathers demonstrate that the tubularity of the feather germ is continuous throughout the entire feather germ, including the vane and the calamus, and between each generation of the feathers.

The essential tubularity of feathers is also revealed by various mutants and developmental accidents (Prum and Brush, 2002). Strong ('02) reports a hybrid dove that was temporarily food deprived during molt which had a calamus-like section in the middle of the vane of each pennaceous feather. This developmental accident demonstrates that the feather vane and calamus are differentiated sections of the same continuous tube.

The recessive feather mutation Porcupine (*pc*) in pigeons (Cole and Hawkins, '30), chickens (Waters, '67; Somes, '90), and Japanese quail (Fulton et al., '82; Cheng and Brush, '84) produces incomplete differentiation and morphogenesis of feather barbs, resulting in brittle tubular, quill-like feathers that cannot unfold into the typical planar form.

Consequences of feather tubularity

How has the tubular organization of the feather germ and follicle contributed to the evolution of innovation in feather diversity? Like the tubular bauplan of the ancestral, triploblastic, bilaterian metazoan, the tubular organization of the feather germ has fostered the evolution of morphological diversity and innovation by providing multiple axes over which differentiation can be organized, and morphogenesis can occur (Fig. 3). The entire feather germ is characterized by three axes that are extrinsic to the tube, or established by reference to landmarks outside the tube itself (Fig. 3A). These include the anterior–posterior
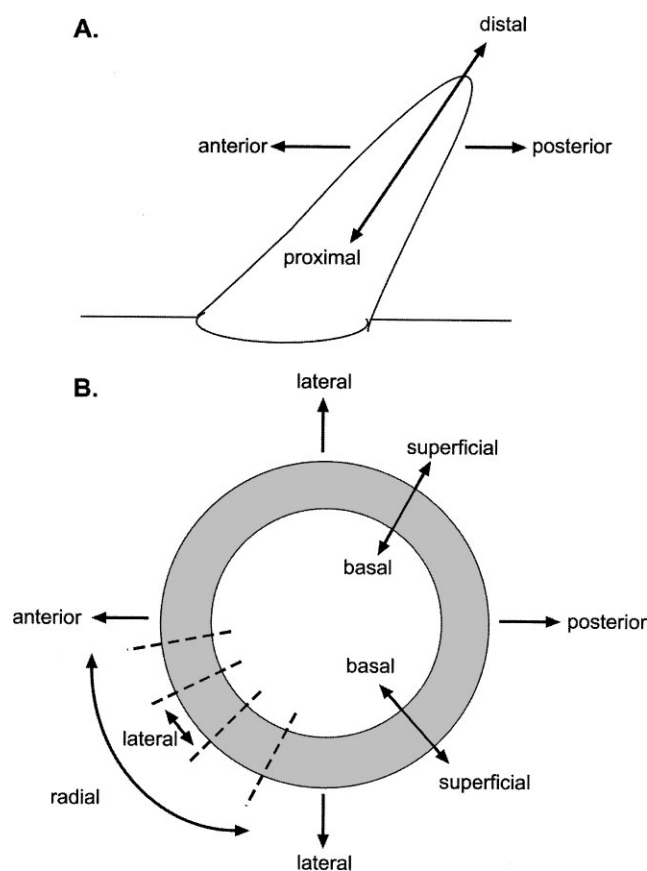

Fig. 3. Diagram of the potential axes of morphological differentiation within the tubular feather germ. (**A**) The feather germ has an anterior–posterior axis, a proximal–distal axis, and a lateral axis (not shown). (**B**) A cross section of the tubular germ shows the additional spatial axes through the cylindrical feather epithelium (gray circle). The anterior–posterior and lateral axes (bold) are in reference to extrinsic landmarks outside the tube (A). The basal–superficial and radial axes are intrinsic to the tube itself. Radial sections of the tubular epithelium create the opportunity for an additional lateral axis that is intrinsic to each section.

axis, the proximal–distal axis, and the lateral (or left–right) axis (Fig. 3A). The tubular feather germ itself is characterized by additional instrinsic spatial axes: the basal–superfcal axis and the radial axes (Fig. 3B). Radial sections of the tube also create the opportunity for an additional lateral axis that is intrinsic to each radial section (Fig. 3B).

Because feathers grow from their base like a hair, temporal differentiation in feather morphogenesis will translate into disto-proximal differentiation in the structure of a single feather (Fig. 3A). This differentiation is seen clearly in most feathers through the differences between the rachis and barbs of the vane and the tubular calamus. Over longer time scales, differentiation over this temporal axis results in diversity of the series of feathers that are grown from a single feather follicle. The feathers grown from a single follicle can be nearly as diverse as all feathers themselves; the follicles on the head of a turkey (*Mjeleagris gallopavo*) grow natal down, juvenal pennaceous contour feathers, and adult feather bristles during the life of the bird (Lucas and Stettenheim, '72).

The feather germ and its various components can also be differentiated laterally. Although the mechanisms have yet to be described, differential recruitment of new barb ridges from the new barb locus to the two sides of the feather vane results in an asymmetrical feather vane (Prum and Williamson, 2001), which is critical to avian flight (Stettenheim, '76). Many feathers also have dramatic asymmetries in shape and structure: for example, the tail feathers of *Menura*, *Cicinnurus*, and *Vidua*, the crown plumes of *Pteridophora*, etc.

Developmentally, the first tubular feather germ grows from the flat feather placode by co-expression of *Sonic hedgehog* (*Shh*) and *Bone morphogenetic protein 2* (*Bmp2*) (Harris et al., 2002). This anterior–posterior axis precedes the subsequent differentiation of the feather germ along the anterior–posterior axis. Later, the tubular feather germ can also be differentiated over a larger number of radial axes (or radii of the tube) to form a series of barb ridges, which become the branched barbs of the feather (Fig. 3B). Barb ridges are created by controlled cell proliferation of columns of intermediate epidermal cells between the basal layer and the superficial layer (the sheath); this process is managed by *Shh* and *Bmp2* signaling in the marginal plate or basal epithelium (Harris et al., 2002). Within radial sections, each barb ridge may itself be differentiated laterally;

for example, differentiation occurs between the distal barbule plates (which create barbules on the rachis side of the barb ridge) and the proximal barbule plates (which create barbules on the side of the barb ridge away from the rachis). This example of lateral differentiation within a barb ridge is not merely anterior–posterior differentiation at the level of a barb ridge, because, in highly asymmetrical flight feathers with a new barb locus displaced laterally from the ventral midline, a new barb ridge must develop distal barbules on its posterior side.

Differentiation within the tubular feather germ also takes place over the anterior–posterior axis of the feather germ, which is present developmentally in the polarized pattern of *Shh* and *Bmp2* expression in the feather placode (Harris et al., 2002). The capacity for anterior–posterior axis of differentiation gives rise to the new barb ridges locus (located posteriorly), helical growth of barb ridges around the tubular feather germ, producing the planar vane, the anterior rachis, and the posterior afterfeather and hyporachis.

The tubular feather germ can also be differentiated across its basal–superficial axis. All vertebrate epidermis consists of stratified layers of cells that proliferate from the bottom and mature vertically. The feather germ proliferates from its base like normal skin, but with the evolution of the tubular feather germ, the plesiomorphic basal–superficial axis of epidermal tissue gains a novel peripheral–internal orientation. Over the scale of the entire feather, differentiation over the basal–superficial axis results in the distinction between the feather sheath and the mature feather itself. Within a barb ridge, differentiation over this axis results in differentiation between the barbule plates and the barb ramus, and between the distal and basal cells within each barbule.

In summary, the tubular organization of the feather germ provides multiple anatomical axes over which differentiation can occur, resulting in a tremendous potential for morphological innovation.

Why tubular?

Given the fundamental role of feather tubularity, one is compelled to ask how the initial tube evolved? Many adaptive explanations for the origin of feathers have been proposed (reviewed in Prum and Brush, 2002); while we can reject the hypothesis that feathers evolved for flight,

numerous other alternative hypotheses remain plausible (Prum and Brush, 2002). However, here I am enquiring more specifically about why tubular organization may be selectively advantageous in response to any number of selective forces.

The tubular feather germ creates an appendage that can grow out of and emerge from the skin without actually increasing the size of the skin itself. With the evolution of periodic pulp caps, the tubular epidermal appendage can continue to grow without continued expansion of the dermal pulp. This proliferative capacity of tubular organization likely provided the first, initial selection advantage to the first feathers and led to the evolutionary fixation and proliferation of these structures around the body.

HIERARCHICAL MORPHOLOGICAL MODULARITY

A striking feature of feather morphology is the hierarchical modularity of feather components and their development (Prum and Dyck, 2003). Morphological modules are serially homologous (or homonomous) replicate morphological entities within the phenotype (Raff, '96). Recently, morphological modularity has been causally associated with the evolutionary origin of diversity because modular components provide opportunities for *independence*, *covariation*, and *interaction* among modules (Müller and Wagner, '91; Raff, '96). Independence of modules provides opportunities for diversification among replicate entities within the phenotype. Covariation and interactions among modules provide opportunities for the creation of *metamodules*—novel, emergent entities in the phenotype (Prum and Dyck, 2003). Hierarchy of modules—either morphological or developmental nestedness of morphological entities—can create additional opportunities for phenotypic complexity, diversity, and morphological innovation (Prum and Dyck, 2003).

Prum and Dyck (2003) have reviewed the hierarchical modularity of plumage morphology and development, and outlined the contributions of this organization to innovations in feather diversity. Essentially, feather morphological complexity exists because of the hierarchical organization in iterated and nested morphological modules. Over each of the axes of differentiation within the tubular feather germ described above, there are distinct, iterated, and frequently nested morphological modules.

For example, the tubular feather germ is divided into the superficial, "deciduous" sheath, the basal layer that forms the persistent feather and the dermis at the center provides nutrition to the developing feather. Within barb ridges, barbule plates develop superficially and the ramus develops internally. Within a barbule plate, the peripheral and internal cells become the distal and proximal cells of the barbule, respectively. This superficial-to-internal modularity is the result of exploiting the plesiomorphic basal-to-superficial stratification of the skin to produce differentiation over a novel axis created by the tubular organization of the feather germ. Radially, the intermediate layer of the tubular feather germ epidermis is compartmentalized into many barb ridges, the rachis ridge, and sometimes the hyporachis ridge. Longitudinally, most feathers are differentiated into a basal calamus and a distal pennaceous vane, plumulaceous tuft, or a combination of the two. Dorso-ventral differentiation in the direction of the helical growth of barb ridges results in the production of a dorsal rachis and a ventral new barb locus, and can also produce an additional ventral hyporachis and afterfeather. With a barb ridge, differentiation between the dorsal and ventral barbule plates creates the opportunity for the pennaceous vane.

Developmental interactions among modules create novel, metamodular structures such as the rachis, whose identity is determined by the fusion of modular barb ridges (Lucas and Stettenheim, '72; Prum, '99; Prum and Dyck, 2003). The role of modular interactions in the production of the rachis is further demonstrated by the development of the afterfeather and hyporachis (Lillie and Juhn, '32; Prum, '99; Prum and Dyck, 2003), and by the classical (Lillie and Wang, '41) and molecular (Yu et al., 2002) experiments which demonstrate that multiple rachi can be produced within a single feather germ.

The morphological differentiation between distal and proximal barbules that forms the pennaceous vane is a consequence of independence of development in the neighboring barbule plates of a single barb ridge and the functional covariance of the distal (hooked) and proximal (grooved) barbules of barbule plates of neighboring barb ridges. However, the actual zippering together of the barbs to create the vane is a physical interaction among the mature barbules from adjacent modular barb ridges to create the metamodular planar vane.

CO-OPTION OF MOLECULAR SIGNALING MODULES

A fascinating molecular component to the evolution of morphological innovation in feathers is the evolutionary co-option of plesiomorphic molecular signaling modules. Gilbert and Bolker (2001) defined a molecular module as an integrated unit of molecular signaling systems that is shared among species. True and Carroll (2002) have reviewed mechanisms of gene co-option in morphological evolution, in which they include derived regulation mechanisms and patterns of expression, and derived protein structure. Here, we are concerned primarily with changes in regulation, expression pattern, and function of the same plesiomorphic genetic signaling modules. True and Carroll (2002) document that gene co-option has greatly contributed to the evolution of morphological complexity and novelty in multicellular organisms.

Although traditional developmental studies of feathers have been pursued for many decades (reviewed in Sengel, '76), molecular studies of feather morphogenesis beyond the stage of placode formation have only begun relatively recently (e.g., Chuong and Edelman, '85; Chuong et al., '90; Nohno et al., '95; Ting-Berreth and Chuong, '96; Morgan et al., '98; Harris et al., 2002; Yu et al., 2002). Taken together, molecular studies of feather development have documented that the genes involved include many of the same paracrine signaling proteins, adhesion molecules, and transcription factors that are broadly involved in vertebrate morphogenesis, particularly of epidermal appendages.

In the first comparative study of the molecular development of avian integumentary innovation, Harris et al. (2002) have documented evidence for repeated evolutionary co-option of plesiomorphic genetic signaling modules in the origin of morphological innovations of feathers. Specifically, in an analysis of alligator, chick, and duck, Harris et al. (2002) demonstrated that the genes for the extracellular signaling proteins *Sonic hedgehog* (*Shh*) and *Bone morphogenetic protein 2* (*Bmp2*) comprise a functional molecular module in the anterior–posterior polarization of avian feather, avian scutate scale, and crocodile scale placodes. These comparative data document clearly the plesiomorphic role of the *Shh–Bmp2* molecular module in archosaurian epidermal appendages. Harris et al. then showed that the *Shh–Bmp2* module function was necessary for the subsequent development of a series of morphological innovations in feather structure, including the origin of the tubular feather germ, the morphogenesis of barb ridges, the development of new barb ridges, the initial fusion of barb ridges to form the rachis ridge, and the subsequent fusion of barb ridges to the rachis ridge (Fig. 4). Thus, a fundamental component of the developmental mechanisms used to polarize archosaurian epidermal appendage
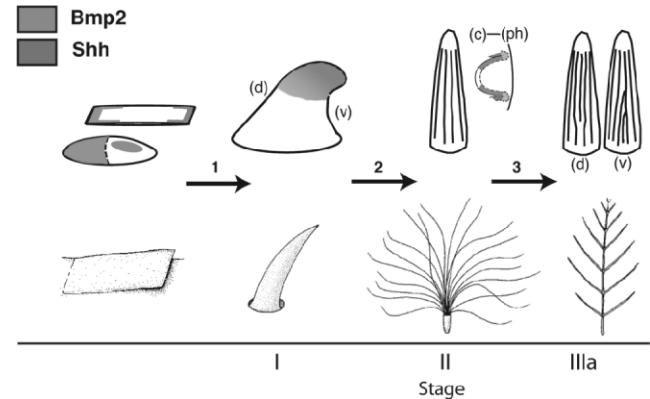

Fig. 4. Pattern of evolutionary co-option of the plesiomorphic *Sonic hedgehog* (*Shh*) and *Bone morphogenetic protein 2* (*Bmp2*) molecular signaling module during the evolution of feather morphological innovations (above) (from Harris et al., 2002), and congruence with the developmental model of feather origin and evolution (below) (from Prum, '99). At four stages of feather development, the *Shh–Bmp2* module has a unique pattern of expression (above, left to right): *Shh–Bmp2* displays an anterior–posterior polarized pattern in the initial placode stage of feathers and archosaur scales; distal co-expression during the development of the tubular feather germ; longitudinal *Shh–Bmp2* expression in the folded marginal plate epithelium between barb ridges during the development of differentiated barbs (see cross-section of a barb ridge inset); and posterior bifurcation and anterior extinction, or cessation, of *Shh–Bmp2* domain expression during the helical growth of a pennaceous vane (Harris et al., 2002). Each of these developmental evolutionary novelties evolved by co-options, or evolutionary reutilizations, of the primitive *Shh–Bmp2* molecular module in a new context. The first, elongate tubular feather (Stage I) evolved from a primitive archosaurian scale by derived distal *Shh–Bmp2* co-expression (Event 1). The first, branched plumulaceous feather (Stage II) evolved by the origin of derived longitudinal *Shh–Bmp2* expression domains (Event 2) that created differentiated barbs from the tubular epithelium of the feather germ. A simple, pinnate pennaceous feather (Stage IIIa) evolved by the controlled dorsal extinction, and ventral bifurcation of the longitudinal *Shh–Bmp2* expression domains (Event 3), producing helical growth of barb ridges, indeterminate barb number, a rachis, serial fusion of barbs to the rachis, and a planar vane. Subsequent events in the development and evolution of feathers (e.g., origins and differentiation of barbules) will require additional mechanistic explanations. Abbreviations: c, central; d, dorsal; ph, peripheral; v, ventral.

placodes was repeatedly, evolutionarily co-opted during the evolution of the morphological innovations of feathers. Harris et al. (2002) and Yu et al. (2002) have provided additional evidence that *Shh* and *Bmp2* also function in the internal polarization of the barb ridges and the morphogenesis of barbules within the barbule plates, which constitutes an additional co-option in the development of an additional morphological innovation.

Gene co-option provides an important molecular mechanism for the origin of morphological innovation in many, diverse morphological systems (True and Carroll, 2002), and feathers provide a premier example of this process. Evidently, for certain functions, it may be easier to evolve mechanisms of developmental "buffering" or regulatory isolation of the diverse functions of a molecular module used in many tissues over many different times in development, than it may be to evolve entirely new genes to produce a morphological innovation. Plesiomorphic signaling modules have already evolved molecular mechanisms to provide controlled morphogenesis, which frequently requires a regulatory balance between morphogenetic and inhibitory signals. Plesiomorphic modules are also, by definition, primitively present in a tissue. Therefore, heritable variations in function of plesiomorphic molecular modules may be readily generated from within the coherent, plesiomorphic developmental processes. Such variations would provide the necessary fuel for subsequent evolution of morphological innovations. For this reason, the evolutionary co-option of plesiomorphic genetic modules observed in many morphological systems may be favored by microevolution, because co-option may be a likely result of selection operating on mutations in development. We also have evidence that *Shh–Bmp2* signaling is critical in establishing the internal peripheral gradient within barb ridges, and is critical to organization of the ramus and differentiation of barbules.

These data are all consistent with the evolutionary co-option, or derived reutilization, of a plesiomorphic signaling system in the evolutionary origin of morphological innovation

Chance and contingency

Harris et al. (2002) provide some additional insight into the role of stochastic processes and historical contingency in the origin of morphological innovations. Harris et al. (2002) documented

that the *Shh–Bmp2* module functions in the morphogenesis of barb ridges through their polarized expression within the marginal plate epithelium which lies between the barb ridges. In whole-mount in situ hybridization staining, *Shh* and *Bmp2* expression exhibits longitudinal stripes along the tubular feather germs (Fig. 5). In the development of embryonic chicken down feathers, which are essentially radially symmetrical and lack a strong anterior–posterior polarization and a prominent rachis, Harris et al. (2002) observed four inherent patterns of deviation from simple longitudinal propagation of expression domains or stripes, which each occurred at low frequency (<5%). Two of these inherent variations in expression pattern—expression domain bifurcation and extinction (or cessation)—are critically necessary to the development of the pennaceous feather, which is the next novelty to be derived. The new barb ridge locus of the posterior side of the feather germ produces new barb ridges and contributes to the indeterminacy of barb number. The new barb ridge locus is the site of the bifurcation in *Shh–Bmp2* signaling domains that produce these new barb ridges. In contrast, on the anterior side of the feather germ, the rachis is formed by the initial fusion of the anteriormost barb ridges to determine the rachis ridge, and subsequent barb ridges fuse to the rachis ridge to create the planar feather vane. The fusion of barb ridges is accomplished by the repeated extinction, or cessation, of *Shh–Bmp2* signaling in the marginal plate epithelium between neighboring barb ridges as they reach the anterior region of the tubular feather germ.

Thus, the organization of *Shh–Bmp2* signaling in a tubular feather germ to undergo posterior bifurcation and anterior extinction creates the morphogenetic mechanisms required to produce the feather vane. These two modes of altered signaling propagation occur at low frequencies (<5%) in radially symmetric, plumulaceous natal feathers of chick. They are maintained as infrequent stochastic variations in modern down feathers that lack any strong anterior–posterior polarization. However, these two mechanisms were co-opted and spatially co-ordinated to create the derived morphogenetic mechanisms necessary for the development of pennaceous feathers. Interestingly, the two other inherent patterns of signal domain propagation—fusion and de novo initiation—produce other morphologies that have not contributed to any morphological innovations to feather diversity.
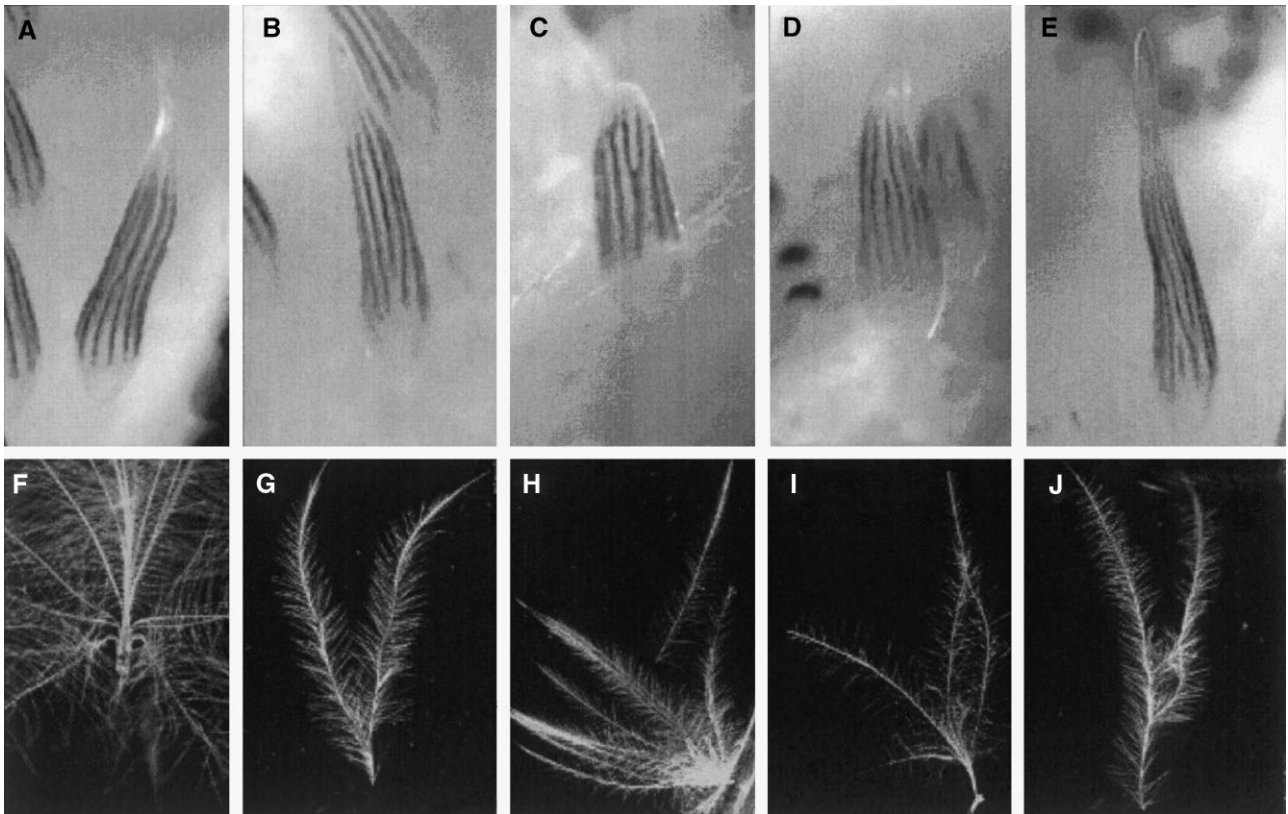
Fig. 5. Inherent variations in the pattern of *Shh* module expression in marginal plate epithelium between developing barb ridges of plumulaceous natal down of chick function in determining barb branched structure (from Harris et al., 2002). These variations have differentially contributed to the evolution of subsequent morphological innovations. (A–E) Feather germs of white leghorn chick embryos illustrate four inherent variations in the propagation of longitudinal *Shh* domains in tubular feather germs. (**A**) Bifurcation of *Shh* expression domain; (**B**) extinction, or cessation, of *Shh* expression domain; (**C**) fusion of *Shh* expression domains; (**D**) de novo initiation of new *Shh* expression domain; and (**E**) simultaneous extinction and initiation events. These stripes of *Shh* expression indicate the folded marginal plate epithelium that differentiates neighboring barb ridges (see inset in Fig. 4). (F–J) Each class of *Shh* expression pattern observed corresponds to an observed phenotype in day-old chick natal down. (**F**) New barb ridge formation resulting in addition of a barb; (**G**) barb fusion in which two barbs are connected proximally to form a single branched barb; (**H**) barb loss in which a free barb is unconnected to the rest of the feather; (**I**) barb division in which a single barb splits proximally into two basal bars; and (**J**) simultaneous barb fusion and barb division. In (G), (I), and (J), the barb shown has been removed from the down feather to illustrate the phenotype of interest. The patterns of posterior bifurcation (A) and anterior extinction (B) are required for the development of the next morphological innovation in feather structure—the rachis and pennaceous feather vane. The patterns of fusion (C) and initiation (D) have not led to any subsequent morphological innovations.

In summary, the evolution of the pennaceous vane occurred through the organized utilization of two of the four modes of *Shh–Bmp2* signaling domain propagation—bifurcation and extinction—whereas the other two were evolutionary dead ends—fusion and initiation.

CONCLUSIONS

The inherent tubularity of the feather germ was the initial morphological innovation that created inherent opportunities for differentiation over multiple spatial axes and fostered the evolution of subsequent innovation. These subsequent innovations evolved through the hierarchical modular differentiation of this tubular structure over these axes. Concurrently, the evolution of the hierarchical, modular morphological innovations of feathers occurred through the repeated evolutionary co-option of plesiomorphic molecular signaling systems. Several feather innovations document the historical and hierarchical contingency of evolution. For example, the formation of the rachis and new barb ridges that characterizes the pennaceous feather evolved through the derived organized utilization of two inherent

patterns of developmental variation found within plumulaceous feathers. In contrast, the two other inherent variations yielded no subsequent evolutionary novelties. This molecular evolutionary co-option likely occurred because plesiomorphic signaling modules provide pre-existing, stable mechanisms to control morphogenesis, and because novelties in the deployment and use of these plesiomorphic signaling systems are likely to create potentially stable new variations that create new opportunities for subsequent morphological novelty.

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