A Hierarchical Model of Plumage: Morphology, Development, and Evolution

RICHARD O. PRUM [1]* AND JAN DYCK [2]
[1]*Department of Ecology & Evolutionary Biology, and Natural History Museum, University of Kansas, Lawrence, Kansas 66045–2454*
[2]*Institute of Population Biology, Universitetsparken 15, DK–2100 Copenhagen, Denmark*

ABSTRACT   Plumage is a complex component of the avian phenotype. The plumage of an individual is composed of numerous hierarchically arranged developmental and morphological modules. We present a hierarchical model of plumage that provides an intellectual framework for understanding the development and evolution of feathers. Independence, covariation, and interaction among plumage modules create numerous opportunities for developmental and evolutionary diversification of feather complexity and function. The hierarchical relationships among plumage modules are characterized by both top-down and bottom-up effects in which properties of modules at one level of the hierarchy determine or influence the properties of modules at lower or higher levels of the hierarchy. Plumage metamodules are created by covariation or interaction among modules at different levels of the hierarchy. *J. Exp. Zool. (Mol. Dev. Evol.) 298B: 73–90, 2003.*   © 2003 Wiley-Liss, Inc.

INTRODUCTION

Plumage is a complex and important component of the avian phenotype. The plumage of a bird consists of thousands of feathers that vary in structure, shape, size, color, and chemical composition (reviewed in Lucas and Stettenheim, '72). Feathers are integumentary appendages characterized by the unique tubular organization of the feather follicle and germ (Prum, '99), and by their capacity for bipinnate structure (Figs. 1, 2) (Lucas and Stettenheim, '72; Prum, '99). The pennaceous contour feathers, flight feathers, plumulaceous downs, disintegrating powder downs, tiny filoplumes, and bristles are some of the extremes of feather shape and structure. The variety of feather structure is determined by variation in the size, shape, and chemical composition of the kerantinocytes that make up the feather.

Avian plumage is renewed throughout the life of the bird through periodic molt. Individual follicles can grow successive feathers that vary radically in structure, shape, and color between molts. For example, during the life of the bird, the follicles on the head of the turkey (*Meleagris gallopavo*, Phasianidae) initially grow plumulaceous natal down feathers, which are subsequently replaced by fully pennaceous contour feathers in the juvenile plumage, and ultimately by simple bristles in the definitive adult plumage (Lucas and Stettenheim, '72). Feather follicle identity is established early in development. Subsequently, the follicle regenerates the appropriate feathers throughout the life of the bird.

Many of these variations in feather structure, shape, and color over the body and the life span are directly correlated with the diverse functions of the plumage in the life of the bird. Feathers are known to function in flight, temperature regulation, water repellency, visual communication, crypsis, sensory detection, sound production, water transport, and other functions (Stettenheim, '76).

Most of what we know about the biology of feathers and plumage comes from extant birds. It is now clear, however, that feathers are not unique to birds. Rather, feathers evolved in a coelurosaurian theropod lineage long before the origin of birds or the origin of avian flight. (Ji et al., '98, 2001; Sereno, '99; Xu et al., '99a, b, 2000, 2001; Padian, 2001; Prum and Brush, 2002). Although the paper repeatedly refers to birds, the plumage phenotype originated in theropod dinosaurs before
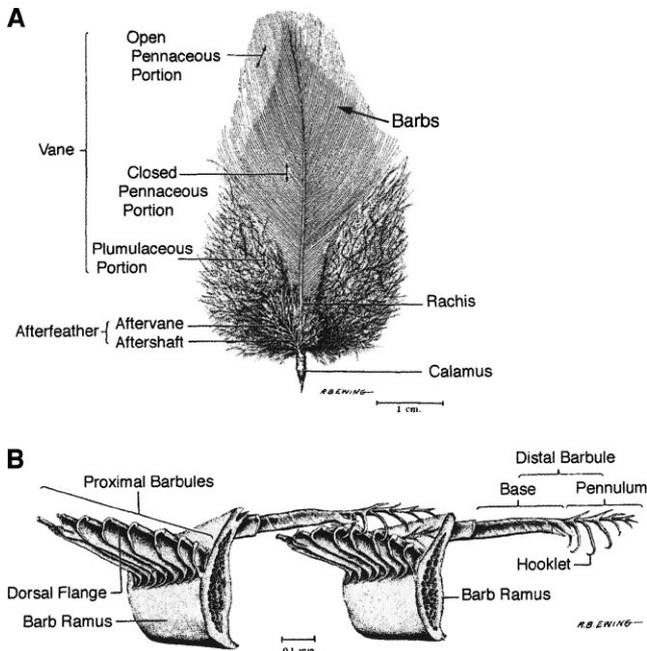
Fig. 1. A. The structure of a typical pennaceous contour feather with afterfeather. B. Cross-section of feather barb rami from the closed pennaceous portion of a feather showing the differentiation between the distal barbules (oriented toward the tip of the feather) and the proximal barbules (oriented toward the base of the feather). The hooks of the pennulum of the distal ends of the distal barbules interlock with the grooved dorsal flanges of the bases of the proximal barbules from the adjacent barbs forming the closed pennaceous portion of the vane. The distal barbules of open pennaceous portions of feathers lack hooked pennula. Both illustrations from Lucas and Stettenheim ('72).

the origin of birds. Thus, the model applies as well to the non-avian plumage phenotype in feathered theropods.

All of the follicles and feathers of an individual bird—or, more accurately, coelurosaurian dinosaur– throughout its life can be conceived of as its *plumage phenotype*. The plumage phenotype is distinct from an individual plumage—a specific coat of feathers of an individual – and provides a new concept for the entire component of phenotype, the subject of feather biology. In this paper, we present a new conceptual model of the development and evolution of the avian plumage phenotype. Intellectually, the model is an application to the plumage phenotype of the concepts of morphological modularity and developmental hierarchy developed by Müller and Wagner ('91), Raff ('96), Wagner (2000), and others. Our goal here is to provide a framework to understand the complexity of plumage, to help define and

differentiate the various levels of analysis within the fields of feather development and evolution, and, most importantly, to focus attention on new questions and motivate future research on plumage development and evolution.

Lucas and Stettenheim ('72: 385, Fig. 241) presented a hierarchical, graphical summary of feather development. Lucas and Stettenheim' s diagram presents the details of the complex development of a single feather in a hierarchical flow chart, an early progenitor of the conceptual model proposed here. (See also Widelitz et al., in this issue for the molecular and developmental events in different stages of feather development.) Here, the goal of our hierarchical model is to provide a heuristic framework that incorporates all components of the plumage phenotype. Further, we propose to integrate into our understanding of feather biology the concepts modularity, hierarchy, and modular interactions (Müller and Wagner, '91; Raff, '96; Wagner, 2000), which have become fundamental to the study evolutionary novelties and developmental evolutionary biology since Lucas and Stettenheim ('72). This hierarchal framework applies to a wide variety of proximate developmental, functional, and, ultimately, evolutionary questions.

Our aims are to propose a unifying framework to organize the breadth of research in feather biology, and to demonstrate that the plumage phenotype is an outstanding model system for understanding the modularity and hierarchy in the development and evolution of biological innovations.

Below, we first introduce the concepts of modularity, hierarchy, metamodules, independence, covariation, and interaction with reference to the plumage phenotype. We then present the hierarchical model of the plumage phenotype. Each section presents the evidence supporting the existence of a module at that level of organization, and a discussion of the relationships and interactions of those modules to other modules in the hierarchy. Finally, we discuss the heuristic features of models for future investigation of the evolutionary developmental biology of feathers.

MODULARITY AND HIERARCHY

Morphological modularity is a fundamental feature of the plumage phenotype that contributes both to its complexity and diversity. Morphological
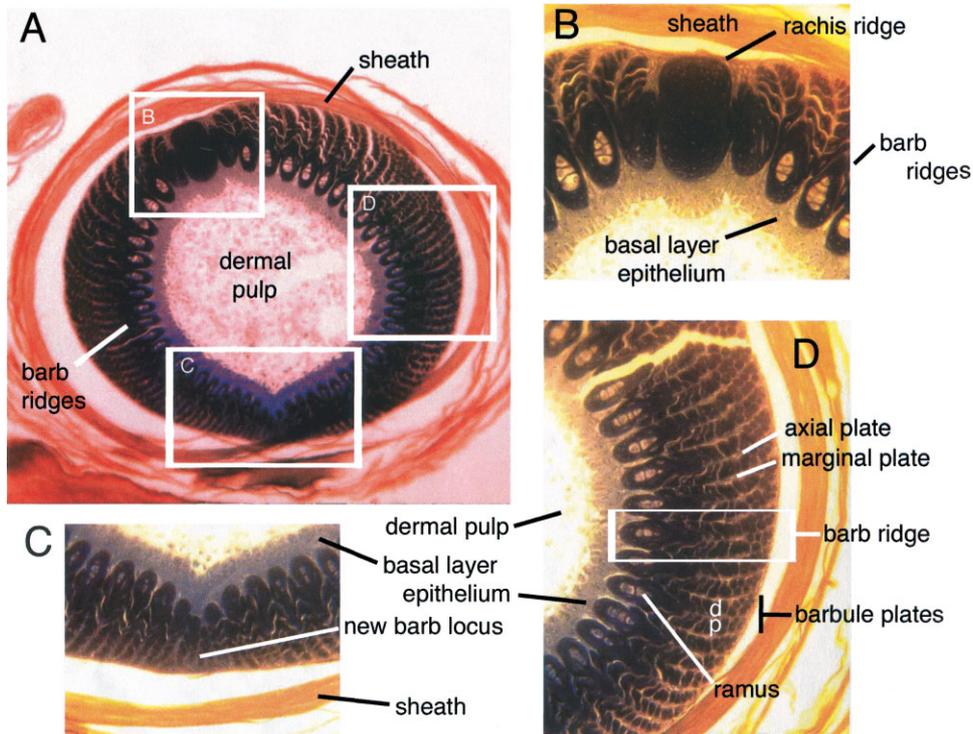
Fig. 2. Histological cross-sections of a developing contour feather germ from a chicken (*Gallus gallus*). A. A cross-section of the entire feather germ with the areas in B, C, and D highlighted with white boxes. B. Close-up of the rachis ridge on the dorsal margin of the feather germ, and the fusion of barb ridges to the rachis ridge. C. Close-up of the new barb locus on the ventral margin of the feather germ where new barb ridges form. D. Close-up of barb ridges from the lateral margin of the feather germ showing the ramus, barbule plates, marginal and axial plate epithelia. The differentiated distal and proximal barbules (Fig. 1B) develop from the distal and proximal barbule plates of each barb ridge. Abbreviations: d, distal barbule plates; p, proximal barbule plates.

modules are serially homologous, or homonomous, replicate morphological entities within the phenotype (Raff, '96). Examples of well recognized morphological modules include limbs, digits, vertebrae, leaves, flowers, and stamens. The plumage phenotype is composed of numerous replicate morphological and developmental modules. For example, the avian integument is organized into the feathered areas, the pterylae or feather tracts, with interposed largely featherless areas, or apteria. Within the pterylae, there are numerous replicate feather follicles. Within a typical pennaceous feather, there are numerous modular feather barbs which themselves are composed a central ramus and numerous replicate barbules (Fig. 1). The plumage phenotype is the sum of these numerous replicate morphological modules over the life of the individual.

A second fundamental feature of the plumage phenotype is that its morphological modules have a *hierarchical organization* (Brush, '93, '96; Prum,

'99; Brush, 2000). Replicate modules are nested within other, more inclusive modules. For example, each barb often includes numerous modular barbules. Most feathers are composed of numerous modular barbs. The plumage phenotype is composed of the hierarchical aggregation of numerous nested, replicate morphological modules within all the feathers of all of the pterylae of integument.

A fundamental question in the evolution of the plumage phenotype is what developmental and evolutionary mechanisms determine the properties and relationships within and among morphological modules? It is clear that developmental mechanisms operating *within* morphological modules can contribute to or determine the properties of the modules nested within it. We call these *top-down effects* because properties at the higher, or more inclusive, levels of the hierarchy determine properties of modules nested at lower levels of the hierarchy. For example, developmental

mechanisms operating at the level of the entire follicle may determine which feather keratinocytes incorporate melanosomes, and thus determine the within-feather pigmentation pattern. Thus, developmental mechanisms operating within a module can contribute fundamentally to determining properties of the modules nested within it, or within a single lineage of the hierarchy of the plumage phenotype. However, the relationships among modules within a single lineage of the hierarchy are not only driven from the top of the hierarchy down. Rather, modules may create *bottom-up effects* in which the properties of lower level modules contribute to or determine the properties of higher level modules that they are nested within. For example, morphology or shape of barbule cells greatly determines whether a feather has a closed pennaceous vane or an open plumulaceous structure. Or, variation in the expression of different feathers keratins within individual keratinocytes will create emergent physical properties at the level of the entire feather. Thus, individual modules can also determine some properties of the larger modules that they are nested within through bottom-up effects or emergent properties.

INDEPENDENCE, COVARIATION, AND INTERACTION

The hierarchical modularity of the plumage phenotype creates intrinsic potential for the development and evolution of additional morphological complexity. These opportunities arise through at least three mechanisms: independence of modules, covariation among modules, and interactions among modules (Müller and Wagner, '91; Raff, '96; Wagner, 2000). These mechanisms operate *among* modules that can be either within individual level or among multiple levels of the hierarchy, and are not entirely mutually exclusive.

Independence of morphological modules provides the opportunity for development and evolution of structural diversity among homologous modules within the plumage (Müller and Wagner, '91; Raff, '96; Wagner, 2000). For example, independence among follicles permits variation in structure, shape, size, and color of feathers on different parts of the body. Independence among barbs within a single feather and of barbules within a barb permits the development and evolution of structural variations that create the pennaceous vane. Developmental independence

and appropriate genetic variation must precede the evolutionary diversification among modules. Independence of modules is not complete, or the integration of the phenotype and its development would breakdown.

Covariation among modules is the consequence of constraints on the independence of modules, or a derived loss of independence among modules. Covariation can create *metamodules* within individual feathers or at other levels of the plumage phenotype. Metamodules are novel, emergent units that are composed of modules from across different lineages or levels of the hierarchy. For example, a distinctly colored plumage patch is a plumage metamodule created by covariation in pigmentation or structural coloration of feathers or parts of feathers, grown from follicles that are distributed across specific portions of the integument, perhaps incorporating different pterylae. Thus, covariation in color patterning of feathers from the follicles within a single or multiple pterylae can create an integrated plumage patch. In some cases, the genetics of covariation within the plumage phenotype are well understood, such as in the Columbian Restriction (*Co*) locus in chickens, which inhibits eumelanin deposition in feathers of the hackle, wing, tail, and feet (Smyth, '90).

Interactions among modules can also create metamodules across lineages or levels of the hierarchy. These interactions can be developmental or physical processes that occur after development is complete. As a developmental example, the rachis ridge is created or specified by the fusion of barb ridges on the anterior side of the feather germ and ultimately becomes the rachis of the feather (Fig. 2B)(Lillie and Wang, '41; Lucas and Stettenheim, '72; Prum, '99; Harris et al., 2002). Thus, the rachis is a unique morphological novelty that develops as a metamodule by an interaction (i.e., fusion) of component modules within the feather germ (Harris et al., 2002). Proximate interactions among covarying modules after the completion of development can also create novel metamodular functional structures. The premier example is the zippering interconnections between the hooked pennula of the distal barbules and the grooved bases of the adjacent proximal barbules that create the closed pennaceous portion of the feather vane (Fig. 1B). Furthermore, the proximate physical interactions among the remiges and rectrices create the physical properties for the wing and tail to form air foils.

A HIERARCHICAL MODEL OF THE PLUMAGE PHENOTYPE

Here we present a hierarchical model of plumage with a description of the modular and metamodular components of the plumage, their major developmental and physical interactions. The model is illustrated in a series of schematic figures that depict the proposed hierarchical relationships (Figs. 3–6). The modules and metamodules are hypothesized based upon the classic descriptions of feather morphology and development (e.g., Lucas and Stettenheim, '72), and upon recent advances in feather development and evolution (Prum, '99; Chuong et al., 2000; Harris et al., 2002).

The presentations of each module below include an anatomical description of the module and its variations; the developmental and anatomical evidence for its recognition as a phenotypic module; a description of its independence from, and its covariation and interactions with other modules; and sometimes discussions of outstanding research questions pertaining to that module. This paper is a linear description of a hierarchical model; without hypertext, it cannot capture the essential organization of the model. As a compromise, we present the descriptions of the modules in the order from the top down–from the most inclusive to least inclusive. Then, we present metamodules at the level of organization of individual feathers, and lastly metamodules at the level of the plumage.

Pterylae and apteria

With few exceptions (e.g., in penguins, Spheniscidae), contour feathers are not continuously
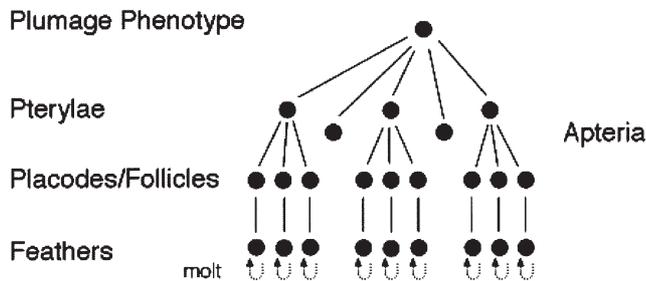
Fig. 3. A schematic diagram of the hierarchical relationships among the morphological modules within the plumage phenotype. The pterylae include numerous feather placodes which each develop into a feather follicle. Each follicle grows a series of feathers, which are replaced over time by periodic molts. Apteria are spaces between pterylae which lack contour feathers or which lack feathers entirely.
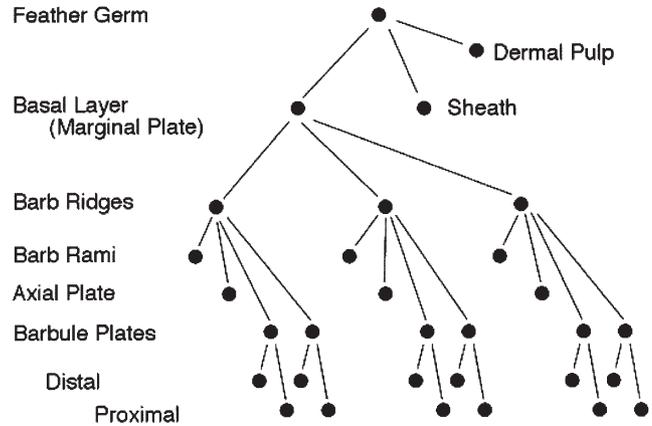
Fig. 4. A schematic diagram of the hierarchical relationships among the basic morphological modules of a feather. The feather germ initially includes the dermal pulp, the sheath (outer epidermal layer), and the basal epidermal layer. The basal layer (including the marginal plate epithelium) controls the proliferation and organization of an intermediate epidermal layer into a series of longitudinal barb ridges. Each barb ridge develops into a single barb ramus and a series of barbule plates, which are separated by the cells of the axial plate. The barbule plates can be differentiated into distal and proximal barbule plates.

distributed over the integument, but are concentrated into specific tracts, called pterylae. Pterylae are interspersed with apteria, which may be entirely featherless or include down feathers. Some apteria occur as unfeathered areas within a single pteryla. The pterylae and apteria are the
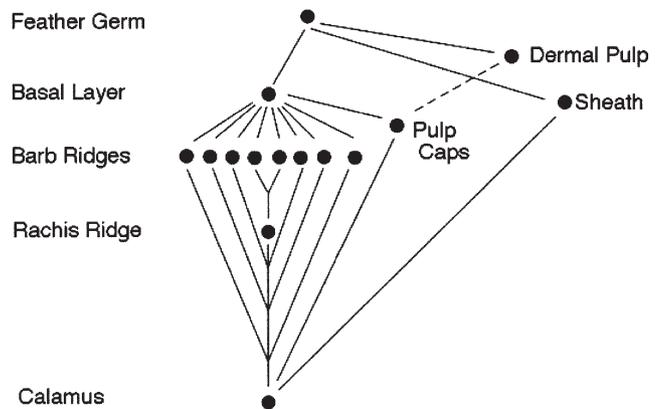
Fig. 5. A schematic diagram of the modular interactions involved in the creation of the feather rachis, a metamodular component of a feather. The rachis is created by the fusion of barb ridges. Subsequent barb ridges fuse to the rachis to create a pennaceous structure. Finally, the rachis ridge and sheath dedifferentiate to become the calamus. A series of pulp caps are produced by the inner-most germinative layer of the basal epithelium through interactions (dashed lines) with the dermal pulp.
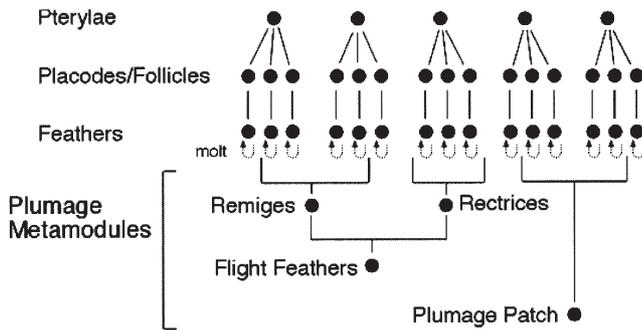
Fig. 6. A schematic diagram of example plumage metamodules created by morphological and developmental correlations among feathers within and among different pterylae. Right– A distinct plumage patch created by correlated coloration among feathers from different pterylae. Left– Remiges (wing flight feathers) comprise a plumage metamodule created from correlation among feathers of pterylae on both wings. Center– Rectrices (flight tail feathers) are a plumage metamodule created by correlation among feathers from a single pteryla. Center Left– All the flight feathers comprise a more inclusive plumage metamodule created by correlations among the remiges and rectrices.

most general, inclusive units in the hierarchy of the plumage phenotype (Fig. 3). The presence of the apteria and regions with other differentiated integumentary appendages (e.g., scutate or reticulate scales on the legs) indicate that the pteryla and apteria are genuine developmental entities with a distinct autonomous identity, and not merely epiphenomena of the process of establishment of feather placode spacing (see below). Recent molecular developmental studies have established that feather placodes and follicles develop in a temporal pattern within a pteryla by a temporal wave of epithelial competence to respond to induction (Noralmy and Morgan, '98; Jiang et al., '99).

Pterylae vary extensively in shape and distribution among different avian families and orders (Lucas and Stettenheim, '72). These variations have been used traditionally in taxonomic studies of birds. Pterylae and the distribution of feathers on the integument have a complex evolutionary history within birds, and certainly preceding birds within other coelurosaurs as well (Prum and Brush, 2002). Apteria are often viewed as merely the areas remaining when the feather development is complete, but as Lucas and Stettenheim ('72) implied, apteria are likely developmental entities with their own modular identity and developmental determination. It is possible that selection at the level of the entire plumage for thermoregulation or communication could be

responsible for the origin of the variation in the distribution of pterylae. However, it is also clear that many pterylogical patterns are functionally redundant, and that all can produce a complete plumage. With the exception of the derived loss of apteria in the cold-adapted penguins, there are few adaptive hypotheses about the evolution of pterylae. For example, nearly all avian species have a prominent ventral apterium which functions proximately in brooding eggs. However, the ventral apterium is not maintained by proximate selection for brooding since many birds independently develop a featherless, edematous brood patch to facilitate incubation. Furthermore, the ventral apterium has not been lost in any lineage of brood parasites (R. B. Payne, pers. comm.). Apparently, there are constraints on the loss of this apterium that have been overcome only in the penguin clade which often brood their eggs on their feet or shanks of the legs. Some species have evolved conspicuous novel apteria through sexual or social selection for display behavior– e.g. the bare bright blue crown skin of *Cicinnurus* and *Diphyloides* birds-of-paradise (Paradiseaidae) and *Perrisocephalus* cotingas (Cotingidae). Interestingly, even though these features have clearly evolved by sexual selection for function in male courtship displays, there is no sexual dimorphism in these apteria. Due to the development of pterylae early in embryogenesis (beginning day 6) before any sexual differentiation has taken place, these derived display apteria are constrained from evolving sexual dimorphism. There may be no available genetic variation for sexual differentiation in components of the phenotype that develop prior to the sexual differentiation. Conversely, many apparently featherless areas are not apteria, but are pteryale filled with small bristle feathers (e.g. the head of the turkey).

Feather placode and follicle

Within the pterylae are the many thousands of feathers (Fig. 3). These feathers initially develop from placodes during the first 6–15 days of incubation. A placode is the epidermal primordium of a feather follicle and feather germ. The first signal to form placode comes from the dermis which determines the site of the feather. The induced epidermis then forms the feather placode by the elongation of epidermal cells. More dermal cells aggregate below the epidermal placode soon after its formation. The epidermal placode and its dermal condensation together develop into the

feather germ and follicle. Feather placodes have been traditionally recognized as morphologically distinct because of the associated dermal condensation (Maderson and Alibardi, 2000), which is also present in avian scutate scales but not in avian reticulate scales or alligator scales (Maderson and Alibardi, 2000).

Although this placode-associated dermal condensation has an evolutionary history that is of substantial interest, the placode exists as a molecular phenomenon among all epidermal appendages as well. It is the site of molecular signals that interact with the dermis and lead to integumentary appendage morphogenesis. The avian reticulate scales, which lack this morphologically defined placode, share distinct placode-specific patterns of gene expression with feather and scutate scale placodes (Chuong et al., '96; Widelitz et al., '99). Furthermore, Harris et al. (2002) documented that the placode of feathers, avian scutate scales, and alligator scales all share an early anterior-posterior polarized pattern of expression of *Sonic Hedgehog (Shh)* and *Bone morphogenetic protein 2 (BMP2)*. The broad distribution of this mechanism of placode specification and development indicates that this plesiomorphic, or primitive, feature was shared by the integumentary appendages of the common archosaurian ancestor of birds and crocodiles. The shared details of the spatially polarized expression of *Shh* and *Bmp2* confirm that the placodes of archosaur scales and avian feathers are homologous, and that the dermal condensation of the avian feather and scale rudiments is subsequently derived in feather and avian scutate scale placodes. Feather placodes have a distinct, uniform hexagonal distribution within the pterylae. Placode spacing is determined by a cascade of inhibitory and activating molecular signaling events that are coordinated by the overlying temporal wave of competence to respond to induction within the pteryla (Chuong et al., '96; Noralmy and Morgan, '98; Jiang et al., '99; Widelitz et al., '99).

After its appearance, the placode elongates into a short bud—a tubular epidermal structure with a central cylinder of dermis. The short bud is the first feather germ. The epidermis of the short bud soon differentiates into the barb ridges that will become the barbs of the first natal down. Very shortly thereafter, the epidermis around the base of the tubular feather germ proliferates and invaginates, forming the cylindrical feather follicle.

Although "feather follicle" sometimes refers only to the socket that holds the developing feather (Lucas and Stettenheim, '72), we refer to the follicle as the organ of feather growth and regeneration. Developmental mechanisms operating at the level of the feather follicle have tremendously influential effects on the individual feather and the entire plumage phenotype. Feather structures develop through differentiation of the tubular epidermis of the feather germ which itself is generated by the internal epidermal layer of the follicle—or follicle collar—through continued induction and nourishment by the papillary dermis in the center of the follicle and germ (Fig. 2A) (Lucas and Stettenheim, '72). Classical experiments bisecting and transplanting part of the epidermal collar and the dermal papilla among feather follicles demonstrate the important role of this organ in determining feather structure (Lillie and Wang, '41; Cohen and 'Espinasse, '61). Examples of feather morphologies that are likely substantially determined by follicle-level processes include the growth of feather shape (Prum and Williamson, 2001), and the growth of within-feather pigmentation pattern (Prum and Williamson, 2002).

The diversity in structure within modular components of individual feathers and among the series of feathers grown from the same follicle demonstrates the flexibility of the developmental mechanisms within a follicle, and indicates that many features of feather structure are determined within lower levels modules of the hierarchy. There do appear to be at least two distinct classes of follicles whose repertoires of developmental mechanisms are quite distinct. Lucas and Stettenheim ('72) documented thoroughly that filoplumes are a distinct class of feathers characterized by a small rachis with a terminal tuft of barbs. Filoplumes develop from a distinct class of follicles that are each associated with and adjacent to a contour feather follicle (Lucas and Stettenheim, '72). In Figure 3, filoplumes are represented by a specific subset of the follicles and feathers within each pteryla. The origin of filoplume placodes is delayed by several days after the associated feather germs have already developed follicles (Lucas and Stettenheim, '72). Even though most feather follicles are capable of growing feathers with a diversity of morphologies, filoplume follicles are distinct in growing exclusively filoplumes. Thus, nested within the most general concept of feather placodes and follicles are the two distinct classes of follicles which grow

filoplumes and all other feathers. The evolutionary and developmental origins of filoplume follicles remains to be investigated.

Feathers

The essential components of the plumage phenotype that develop from follicles are the feathers themselves. The development of the first natal feathers is crucial to the development of the feather follicle itself, but subsequently the feathers are sequentially replaced throughout the life of the bird through periodic molt (Fig. 3). Subsequent feathers grown from a single follicle are hypothesized to be temporal iterations of a homologous tubular feather germ. The feather is regenerated by the follicle through proliferation and differentiation of the follicle collar. The continuity of the tubular epidermis between feather generations is demonstrated by the physical interconnection between the calamus and the distal tips of the initial barb ridges of subsequent feather generations. This feature is easiest to observe in the natal down feathers connected to the tips of the first juvenile contour feathers (Lucas and Stettenheim, '72; e.g., Figs. 229–231). Based on experiments with regenerating plucked feathers, Cohen and 'Espinasse ('61) argued that a feather develops from the elongation of the follicle collar, and is not generated by the collar. Their conclusions were inherently based upon the damage that comes from experimental plucking of a mature feather, and further leaves the question unanswered as to how the follicle repeatedly regenerates the feathers of the appropriate phenotype. We agree with Lucas and Stettenheim ('72) that the collar refers to that enduring epidermal tissue at the base of the follicle that supports regeneration of subsequent feathers during molt.

The identity of a follicle occurs early in its development, and is retained permanently. Consequently, a follicle consistently generates appropriate feathers with each molt. Independence among different follicles within and among pterylae provides the opportunity for the evolution of the functional diversity within the plumage.

Independence and decoupling of the development of feather generations grown from a single follicle permits the extraordinary morphological and functional diversity through the life of the bird. Such diversity includes the differences between natal down—the first plumulaceous plumage to cover the integument of hatchling birds— and subsequent feather generations. The exhibition of different plumage coloration with distinct patches in different parts of the year is another example of developmental independence among feather generations.

The feather and feather germ consist of nested sets of modular developmental and evolutionary units (Fig. 4).

Dermal pulp

The center of the feather germ is occupied by the dermal pulp (Fig. 2). Although not usually considered a component of the plumage, the dermal feather pulp is a critical component of the feather germ. The pulp supplies nutrients to the developing feather epidermis. Also, melanocytes and pigment cells that transfer pigments to the feather epidermis migrate into the feather germ from the central dermal pulp. The dermal pulp is produced continuously but is reabsorbed periodically (Lillie, '40; Lucas and Stettenheim, '72). This process occurs with the periodic production of pulp caps by the germinative, or innermost, layer of the basal layer epithelium (see **Basal Layer** below). Although the pulp caps consist of keratinized epidermal cells, the interactions between the dermal pulp and the germinative basal layer are critical to the development of this modular component (Fig. 5).

Sheath

The sheath is a distinct modular component of the feather germ that is produced by the outer epidermal layer of the feather germ (Figs. 2, 4) (Lucas and Stettenheim, '72). The sheath forms a deciduous outer layer to the feather germ that permits the feather germ to emerge smoothly from the follicle, protects it during development but then flakes apart, and falls off after the feather components have completely matured. The sheath is ultimately subsumed by the production of the calamus at the base of the feather (see below).

The sheath is distinct in that it is composed of α keratins, in addition to the β keratins that exclusively comprise the rest of the components of the mature feather (Maderson and Alibardi, 2000). Maderson and Alibardi (2000) imply that the pattern of vertical alternation of α and β keratins in the feather germ could be homologous with the vertical stratification of keratin classes in the scales of shedding lepidosaurs. However, these protein expression features are clearly convergent between birds and lepidosaurs (Prum and Brush,

2002). As elsewhere in the reptilian integument, the expression of α-keratin in the feather sheath is associated with its flexible properties and its deciduous function.

As the cells of the sheath become a component of the developing calamus, the sheath ceases to be a distinct entity and cannot be identified histologically as a distinct layer of the β keratin calamus (Lucas and Stettenheim, '72: 381). This observation reinforces the conclusion that the sheath is not a lineage of α keratin expressing epidermal cells continuous through all feather generations, but a modular component of the feather germ that is regenerated with each new feather in response to molecular signaling to differentiate the outer epithelial layer.

Basal layer

The basal layer of the feather germ does not become a conspicuous component of the mature feather, but it plays a crucial role in the morphogenesis of the barb ridges, barbules, barb rami, the rachis, and pulp caps of the feather (Fig. 2). The basal layer epithelium of the feather germ is homologous with the general basal layer of the epidermis (Lucas and Stettenheim, '72). It forms the boundary between the barb and rachis ridges, which develop into the mature feather, and the central dermal pulp (Fig. 2B-D). Due to the tubular configuration of the feather germ, the basal epidermal layer is the internal or central-most epidermal layer of the feather germ, and the intermediate and outer layers are peripheral to it.

The barb ridges are the longitudinal masses of intermediate epithelium cells that are bordered internally by a layer of cells that comprise the basal layer (Fig. 2). The basal layer forms a series of folds toward the periphery of the feather germ that separates adjacent barb ridges with a double cell layer. It also forms an intervening single cell layer that cover the internal surface of the barb ridge. The folded portions of the basal layer are also referred to as the marignal plate, or marginal plate epithelium. Lucas and Stettenheim ('72) summarize the confusion among classic studies over the role of the basal layer in barb ridge formation. Lucas and Stettenheim ('72), Strong ('02), and Hosker ('36) hypothesized that the basal layer cells rearrange themselves around the bulge of intermediate epidermal cells of the barb ridges, and that they may be forced into position by the dermal pulp. Griete ('34) alternatively proposed that the axial organization of the barb ridges originated first, and that the basal layer cells

differentiated in situ. Most recently, Yu et al. (2002) hypothesize that the intermediate layer is "cleaved" into barb ridges by the marginal plate. All these models posit that the intermediate layer itself precedes the formation of the barb ridges, and that the process is essentially the subdivision of this intermediate layer.

Recently, Harris et al. (2002) have described the developmental roles of *Sonic Hedgehog (Shh)* and *Bone morphogenetic protein 2 (Bmp2)* expression in the basal layer epithelium and in the creation of barb ridges. Early in the formation of a barb ridge, *Shh* is generally expressed in all marginal plate cells in the folded portions of the basal layer between barb ridges, whereas *BMP2* is expressed in a restricted zone at the periphery of the fold of the marginal plate epithelium. During the formation of a new barb ridge, proliferation of barb ridge cells begins at the site of the peripheral fold in the marginal plates of neighboring ridges (Harris et al., 2002). The lineages of intermediate cells that form the barb ridges initially proliferate between the marginal plate epithelium and the outer epithelium (or sheath). As they proliferate, a notch forms in the peripheral fold in the marginal plate. The new barb ridge then expands balloon-like through cell proliferation into the basal layer, and is flanked by a new set of *Shh* expressing marginal plate cells. Similarly, the fusion of barb ridges (or the loss of distinct differentiation between neighboring barb ridges) is initiated peripherally through the gradual peripheral to central retraction or diminishment of the marginal plate epithelium (Harris et al., 2002). Further, Harris et al. (2002) indicate that polarized *Shh-Bmp2* signaling within the marginal plate epithelium orchestrates the proliferation of the barb ridge cells between the marginal plate and the outer epithelial layer.

These data from Harris et al. (2002) are incompatible with the classic notion that barb ridges are formed by the subdivision of a pre-existent intermediate epidermal layer (e.g. Lucas and Stettenheim, '72: 376. Fig. 238). Rather, the intermediate layer that becomes the feather is created through controlled morphogenesis orchestrated by signaling from the basal layer. Harris et al. (2002) hypothesize that *Shh* plays a role in fostering proliferation of barb ridge cells, and that *Bmp2* plays a role in controlling *Shh* signaling, limiting proliferation, and ultimately fostering cell maturation. The basal layer and marginal plate signaling have an additional crucial role in the morphogenesis of the rachis and pennaceous

structure (Harris et al., 2002). Thus, the same developmental mechanisms that create the differentiated intermediate epidermal layer of the feather germ are responsible for morphogenesis of many of the diverse structures that are created from this layer.

The germinative layer is the inner-most component of the basal epidermal layer (see Lucas and Stettenheim, '72: 384–385), and forms the keratinized pulp caps through interactions with the dermal pulp and the process of dermal pulp reabsorption. The other basal membrane cells, including the marginal plate cells, ultimately undergo apoptosis (Yu et al., 2002), and do not become a component of the mature feather. Yu et al. (2002) hypothesize that apoptosis of the marginal plate cells permits the barbs to open up. Strictly speaking, however, only those keratinocytes that establish tight cell connections (through desmosomes or other structures) during keratinogenesis have the potential to remain interconnected as the feather emerges (Mantulionis, '70; R. O. Prum, pers. obs.). For example, the cells of adjacent barbules on a barb are immediately adjacent to one another without any intervening layer of apoptotic cells (Fig. 2D), yet they show no problem opening up upon expanding. Thus, apoptosis appears to be the way of ultimately eliminating the basal layer cells, but it does not provide a crucial mechanism for the separation of barbs themselves (i.e. experimentally suppressing apoptosis of marginal plate cells should not produce a feather that could not open).

Barb ridge—barbs, ramus, barbules, and axial Plate

The fundamental, differentiated, modular unit of the intermediate epidermal layer of the feather germ are the barb ridges (Figs. 2, 4)(Lucas and Stettenheim, '72). Barb ridges first develop on the anterior (or dorsal) side of the initial feather germ (Lucas and Stettenheim, '72), but in at least some pterylae of the chick (*Gallus gallus*) barb ridge origin is simultaneous around the entire feather germ (R. O. Prum, pers. obs.). The barb ridges have complex modular components nested within them: the presumptive barbs ramus (the main shaft of the barb); the paired, peripheral barbule plates, which develop into the paired barbules; and the axial plate (Figs. 2, 4). Interactions among barb ridges (mediated by basal layer signaling) are also responsible for the creation of additional meta-modules including the rachis and the calamus (Fig. 5).

Within the developing barb ridge, the more central cells develop into a series of longitudinally interconnected cells that form the ramus, or shaft of the barb (Fig. 2, 4). The morphology of the barb ramus is complex, multicellular, and differentiated into cortical, medullary, and central layers (or pith). The outer, more peripheral cells within the barb ridge differentiate laterally into a pair of peripheral barbule plates and the central axial plate. The axial plate tissue separates the two barbule plates during their development and then disintegrates (Lucas and Stettenheim, '72). The barbules develop from a single row of cells in a single layer of feather germ cells. The more basal, innermost cells become the base of the barbule and ultimately connect the barb ramus to form the branched structure of the barb (Fig. 1B). The more peripheral cells become the elongate distal cells of the barbule pennulum (Fig. 1B). As the barbule keratinocytes elongate, they are constrained from growing outward by the feather sheath, so they grow upward within the feather germ. This physical pattern is well illustrated in Fig. 239 of Lucas and Stettenheim, '72. Although barbules develop from a single row of cells, the distal elongation of the barbules means that a horizontal cross-section of a maturing, keratinizing barb ridge will sample cross-sections of numerous barbules, with the oldest toward the center and the youngest toward the periphery.

The axial plate is composed of the barb ridge cells along the central axis of the barb ridge between the two barbule plates (Fig. 2D). The axial plate cells do not form a component of the mature feather. According to illustrations of Yu et al. (2002), the axial plate cells also die by apoptosis.

Barbule morphology is a very conspicuous component of feather morphology because barbules can have important bottom-up effects on the structure of the feather vane. Plumulaceous feathers are characterized by barbules with a series of simple cells within the pennulum (often with nodal prongs at cell junctions that create space-filling tangles among barbule filaments), and little lateral differentiation among barbules. In contrast, a feather with a planar pennaceous vane is characterized by strongly differentiated barbules on either side of the barb ridge (Fig. 2D). The barbules that extend from the ramus toward the tip of the feather—called distal barbules—have conspicuous hooks on the pennulum cells, whereas the barbules that extend toward the base of the entire feather—called proximal

barbules—have a prominent groove in the cells at their bases (Fig. 1B). The differentiated proximal barbules reach over the obverse (outer) surface of the planar vane and the appropriately oriented hooklets interact with the grooved bases of the proximal barbules to create the coherent structure of the pennaceous vane. This differentiation is a consequence of decoupling the development of the paired barbule plates within a cell layer of the barb ridge from one another.

Interestingly, the first cells in the barb ridge to mature are the peripheral barbule plate cells and ultimately the more central ramus cells. It has been hypothesized that this sequence is derived from a developmental mechanism that facilitates nutrient supply to the peripheral tissues first (Prum and Brush, 2002). If the central tissues of the ramus were to keratinize first, it would prevent nutrient supply to the peripheral cells. The importance of nutrient transport and pigment cell transport is demonstrated by the existence of numerous gaps between barb ridge cells until they finally begin to keratinize.

Little is known about the molecular mechanisms of barbule development and differentiation. Harris et al. (2002) hypothesize that this gradient in differentiation is established and controlled by polarized *Shh-Bmp2* signaling in the adjacent marginal plate. Yu et al. (2002) establish that *Bmp2* signaling switches from the peripheral fold of the marginal plate epithelium to the peripheral portions of the paired barbule plates later in development. As hypothesized by Harris et al. (2002), localized *Bmp2* signaling in barbule plates later in development may play a similar role in controlling the response to the persistent *Shh* signaling, which favors cell proliferation, and fostering the maturation and keratinization of barbule cells. Nothing is known about the mechanism responsible for the differentiation of distal and proximal barbules, which must depend upon establishing a rachis vs. new barb locus side identity to the two barbule plates within a barb ridge. Further, no primary histological descriptions or experimental studies have been done on the development of barbs in feathers that lack barbules (such as the display plumes of certain egrets *Casmerodius*).

Rachis

In a pennaceous feather, the rachis is the main shaft of the feather vane to which the barbs are fused at their bases. The rachis has long been described as developing from a rachis, or rachidial, ridge (Lucas and Stettenheim, '72). This special name has contributed to the erroneous notion that the rachis is a distinct entity from the barb ridges (Maderson and Alibardi, 2000), sometimes erroneously hypothesized to be homologous with the central ridge of a reptilian scale. A simple inspection of the diversity of feather morphologies, however, demonstrates that the identity of the rachis ridge is established during development. The distal tip of nearly every pennaceous or plumulaceous feather is comprised of a set of equivalently sized barbs that lack a presumptive rachis (e.g. Lucas and Stettenheim, '72: Figs. 229–231). Describing rachis formation in chicken feathers, Lucas and Stettenheim ('72:371) wrote "two to four barb ridges in the middorsal region of the blastema [or feather germ] fuse at their proximal ends, thereby creating the apex of the rachis." Harris et al. (2002) have further established that the rachis ridge of the pennaceous embryonic duck rectrix is established through the initial fusion of barb ridges on the anterior side of the feather follicle. The initial fusion of barb ridges to form the rachis (and the later fusion of barb ridges to the rachis) proceed from the periphery of the marginal plate to the center (Harris et al., 2002). Lastly, the distinct or singular identity of the rachis ridge has been falsified by classical experimental developmental studies in which multiple rachi are formed within a single feather germ by experimental bisection of a regenerating feather follicle (Lillie and Wang, '41; Cohen and 'Espinasse, '61).

Once established, the rachis ridge develops a distinct identity with a distinct internal structure, including a medullary layer and pith. After the rachis ridge is formed initially, barb ridges continue to fuse to it as they reach the dorsal side of the feather follicle by helical growth (Lucas and Stettenheim, '72; Prum, '99). Barb ridges that fuse to the rachis constitute the lateral cortex of the developing rachis, as shown elegantly by Lillie and Wang ('41). In this fashion, helical growth of barb ridges around the follicle, rachis ridge establishment, serial fusion of barb ridges to the rachis ridge, and continued origin of new barb ridges on the posterior (ventral) new barb locus (Fig. 2C) create the complex structure of the pinnate feather with a planar vane (Lucas and Stettenheim, '72; Prum, '99; Prum and Williamson, 2001).

Occasionally, the rachis bears barbules in the internodes between barb fusions (e.g. *Pavo* rectrices). Apparently, the barbule developing

capacity of the peripheral tissue of the barb ridge is retained after fusion to the rachis, until the next fusion event. This phenomenon reaches its extreme in the racket-tailed parrots (*Prioniturus*, Psittacidae) in which the rachis bears barbules for the length of the intermediate barb-free zone of its racket-shaped central rectrices (Bleiweiss, '87). Interestingly, the development of these rachis-born barbules has never been described histologically.

Toward the base of the vane as the last barb ridges fuse to the rachis, the rachis enlarges in size providing additional structural support to the feather.

Afterfeather and hyporachis

The afterfeather is a ventrally oriented feather vane that grows simultaneously from the same feather germ and follicle (Fig. 1A). The vane of the afterfeather is usually plumulaceous and much smaller in size than the main feather vane, but in cassowaries (*Casuarius*) and emus (*Dromaius*) the afterfeather is identical in size and morphology to the main vane. The afterfeather has a patchy distribution among modern bird orders, but it is invariably absent in remiges and rectrices (Ziswiler, '62; Lucas and Stettenheim, '72). The hyporachis (the shaft of the afterfeather) and the afterfeather are formed by a duplication of the helical growth and serial barb ridge fusion that creates the main vane that is oriented toward the posterior (ventral) margin of the follicle and feather germ (Lillie and Juhn, '38; Ziswiler, '62; Lucas and Stettenheim, '72). Accordingly, the hyporachis ridge is established by the fusion of barb ridges on the posterior (ventral) margin of the follicle. As a consequence of posteriorly-oriented helical growth by barb ridges in a posterior quadrant of the follicle, the posterior locus of new barb formation duplicates into two laterally displaced centers of new barb ridge formation (Lillie and Juhn, '38; Ziswiler, '62; Lucas and Stettenheim, '72). Prum ('99) hypothesized that the afterfeather and hyporachis are secondarily derived, because the mechanism of hyporachis and afterfeather formation is a duplication of the mechanism that creates the main vane.

Calamus

The calamus is the tubular base of the feather. The end of the rachis (if present) and the beginning of the calamus is marked by the superior umbilicus. As elegantly stated by Lucas and Stettenheim ('72), the superior umbilicus is the opening through which the dermal pulp, that is wrapped in the feather vane and will be released as the feather unfolds from the sheath, passes into the inside of the keratinized tube that becomes the calamus. Calamus formation is characterized by the loss of differentiation between the outer and intermediate epidermal layers. The sheath unites with the calamus and ceases to be a distinct, α keratin expressing cell lineage. During growth of the feather vane, the pulp caps are only weakly keratinized and are typically destroyed as the feather emerges from the sheath. Within the calamus the pulp caps become permanent structures that are integrated into the feather.

Feather keratinocytes

The mature feather is composed of the feather type β keratins produced by the keratinocytes of the developing feather germ and the pigments they contain (Brush, '78, '93). Feather keratinocytes are the ultimate modular, cellular components of all the larger units of the feather. There are a number of critical developmental features that are determined at the level of the keratinocytes. These include, but are not limited to, cell number, cell size, cell shape, the types and proportions of keratins to be expressed, whether or not to incorporate eumelanin or phaeomelanin granules, whether to accept lipid soluble carotenoid pigments, and the internal structure of keratin deposition within the cell. Little is known about the mechanisms of feather β keratin synthesis and self assembly within feather keratinocytes beyond a few transmission electron microscope studies (Mantulionis, '70; Bowers and Brumbaugh, '78; Alibardi, 2002).

The number, size, and shape of any keratinocytes will critically effect how the larger units of the hierarchy function in the completed feather. Although the diversity of feather keratins and the variation of expression between pennaceous and plumulaceous feathers (or even pennaceous or plumulaceous portions of a single feather) support the hypothesis that variation in feather keratin expression is functional or adaptive (Brush, '78, '93), we do not yet know enough about the physical properties of these keratin variations to test this hypothesis critically.

The emergence of the feather from the tubular sheath to form the planar vane requires that the

mature but ensheathed barbs and barbules must be "spring-loaded" and ready to assume their appropriate angles (see *Pennaceous Vane* below) ('Espinasse, '39; Prum and Williamson, 2001). How the angles of the barbs to rachis and the barbules to the ramus are specified by developing keratinocytes is a fundamental question that has never been addressed. We do not know of any previous hypotheses addressing how these physical properties are determined during development. Apparently, the deposition of keratin within cells, the cell shapes, and the junctions among cells are appropriately engineered within the cylindrical feather germ so that upon emergence these structures can assume a coherent, functional shape. This keratin engineering is an additional, potentially important function of diversity of β-keratin composition of feather components.

Color of feather keratinocytes

The color of feather keratinocytes can come from pigments or structural color (Lucas and Stettenheim, '72). Pigments in birds include melanin, carotenoids, and others. Feather melanins come from melanocytes of neural crest origin (Lecoin et al., '98) that migrate into the developing feather germ from the dermal pulp of the feather germ (Greite, '34; Watterson, '42; Strong, '02). These melanocytes extend pseudopodia among the epidermal cells of the barb ridges, and transfer fully developed melanosomes to the developing feather keratinocytes (Greite, '34; Watterson, '42; Lucas and Stettenheim, '72; Strong, '02). Melanosomes are actively taken into the keratinocytes by phagocytosis (Greite, '34; Watterson, '42; Lucas and Stettenheim, '72; Jimbow and Sigiyama, '98; Sharlow et al., 2000), and are incorporated into the keratin of that cell as it completes its development and dies.

Feather carotenoids are introduced into feather keratinocytes in a solution of fat globules that come from the blood supply in the dermal feather pulp (Lucas and Stettenheim, '72; Brush, '78). The precise mechanism by which fat soluble carotenoids travel from the blood vessels of the dermal pulp into specific keratinocytes of the feather germ has not been described (A. H. Brush, pers. comm.). Although the capacity for patterning in carotenoid pigments within feathers is not as precise as for melanin pigments, differential distribution of carotenoid pigments within and among feathers of the body does demonstrate

that feather keratinocytes have the capacity to selectively absorb carotenoids, and in some cases to differentiate among some different classes of carotenoids.

A complex of features determines whether a mature keratinocyte produces a structural color—i.e., creates visible hue as a result of interactions of light with the physical structure of the cell. Iridescent structural colors are generally produced by constructive interference, or coherent scattering, of light by layers of melanin granules in feather barbules (usually only the distal barbules which cover the obverse surface of the feather) (Dyck, '76; Durrer, '86). These hues are determined by a number of factors: the size of the melanosomes produced by melanocytes, final spatial arrangement of the melanosomes within the barbule keratinocytes, and the amount of keratin deposited between the melanosomes. In contrast, typically noniridescent colors are produced by coherent scattering of light by the spongy keratin-air matrix within the mature medullary cells of feather barb rami (Prum et al., '98, '99). In these feathers, the hue is produced by selective constructive interference of specific wavelengths of light waves, which are determined by the size and spatial distribution of the air-bubbles (originally cytoplasm of the keratinocyte) within the medullary ramus keratinocytes (Prum et al., '98, '99). Little is known about the development of color producing, spongy medullary barb keratin (Auber, '71, 72).

The pennaceous vane

The closed, pennaceous vane of a contour feather is a complex and functionally important feather metamodule (Figs. 1, 5). The vane itself does not exist during development but is created only after the feather emerges from its tubular sheath and expands to assume a planar shape. Only at this time, after keratinocyte development is completed, do the barbs expand to assume an appropriate angle with the rachis, and the barbules expand to interconnect with one another, and create the closed vane. The metamodular, closed pennaceous vane is a consequence of numerous diverse and covarying features at all levels of the follicle and feather hierarchy, from keratinocyte to follicle. For example, these details must include keratinocyte size and shape, proximal and distal barbule differentiation, basilar-outer differentiation within barbule plates, differentiation of the barbule plates from the

ramus, the helical growth of barb ridges, creation of the rachis, subsequent barb ridge fusion to the rachis, and the predetermined spatial relationships assumed by these components upon emergence from the tubular sheath.

Although feathers with a closed pennaceous vane constitute only one of the many structural classes of feathers, they are functionally, critically important, given their role in flight, creating the contour of the body and the outward appearance of the plumage.

Within-feather pigment pattern

Another important feature of the pennaceous feather vane is pigmentation patterning. There is an enormous variety in within-feather pigment patterns that contribute importantly to the plumage phenotype. Prum and Williamson (2002) have provided the first unified models of the development of within feather pigmentation patterns. Their analyses indicate that barb ridge identity plays no role in the determination of feather pigment patterning. Rather, it appears that spatial position within the feather germ and time during development determine whether particular keratinocyte cells will incorporate melanin pigments and how they will contribute to overall feather pigment patterns. Applying standard reaction diffusion equations to a computer model of the growing feather germ, Prum and Williamson (2002) documented that many feather pigment patterns can be simulated by hypothesizing gradients of activating and inhibiting morphogens within the feather germ and follicle. They were also able to document detailed congruence between the dynamics of the models and the documented variation in pigment patterning. Experimental research on the molecular basis of feather pigmentation patterning is required to test these models. Traditional experiments on avian embryos may be limited because of lack of strong pigmentation patterning in most natal downs. Although barb ridge identity itself is not critical to the establishment of feather pigment pattern, other keratinocyte identities do play a crucial role. Thus, in many birds the distal barbules that cover the outer (obverse) surface of the pennaceous feather vanes are more heavily pigmented than the proximal barbules (e.g., Strong, '02). This detailed patterning in pigment deposition requires patterning mechanisms that distinguish between distal and proximal barbule plate cells that are separated only by a single layer of axial plate cells (Fig. 2D).

Plumage level metamodules

In addition to the modular units of the plumage hierarchy, the plumage phenotype is composed of additional metamodules that include multiple feathers from different or multiple pterylae (Fig. 6). Examples include any group of feathers or parts of feathers that combine to form a distinctly colored plumage patch, such as the wing bars of a wood warbler or the epaulets of a Red-winged Blackbird (Fig. 5). The remiges and rectrices covary in structure, shape, size, coloration, and timing of molt to create the air foils that function in flight. Additional components of covariation among metamodules—e.g., between the wings and the tail—can create additional hierarchical relationship among metamodules. Thus, the left and right sets of remiges, all remiges, all rectrices, and all remiges and rectrices together (i.e., all flight feathers) comprise a complex nested set of plumage metamodules (Fig. 6). A complex example of a plumage metamodule comes from the remiges and rectrices of the Golden-winged Manakin (*Masius chrysopterus*), which are all black with brilliant yellow inner vanes. These distinctly patterned flight feathers appear black when the bird is perched, but they combine to create a brilliant visual display when the wings and tail are spread during the courtship display behavior (Prum and Johnson, '87).

Origin and evolution of plumage meta-modules requires genetic covariation among the appropriate components of the plumage hierarchy, and likely subsequent selection on the emergent functions of these plumage metamodules. Recent research on the evolution of plumage patterning in orioles by Omland and Lanyon (2000) documents the dynamic pattern in the evolution of the expression of pigment pattern metamodules within this clade. Many distinctive plumage patches, such as wing bars, epaulets, hoods, tail patches, etc.– have evolved convergently and been lost repeatedly within oriole phylogeny. This evolutionary pattern indicates that these plumage color patch metamodules are plesiomorphic entities that have been retained, redeployed, and replaced frequently during the history of sexual and social selection on oriole species.

Molt and feather wear

Molt is the periodic replacement of feathers within each follicle. Within this model, molt is the renewal of an entire plumage through the growth

of serially homologous replacement feathers. Most feathers are molted once a year, but timing may vary from once every two years to two to three times a year. Normal molt maintains the functional continuity and integrity of the plumage during the process of complete or partial plumage replacement. Molt requires an elaborate coordination, or covariation, in developmental timing among feather follicles throughout the body. The phenomenon of fright molt (or *Schreckmauser*), in which all the feathers are dropped from their follicles within a few minutes of a severe disturbance (Dathe, '55; Lucas and Stettenheim, '72), demonstrates that an important primary function of the feather follicle is to retain the feather between molts. (A Kansas ornithology student once observed entirely featherless chickens running around minutes after a tornado hit a neighbor's farm. Everyone concluded that the tornado had blown the feathers off.) The timing of feather replacement is under strict hormonal control. Maintenance of flight function during molt requires the replacement of remiges and rectrices in a series without completely compromising the function of the entire airfoil. Different groups of birds have evolved different sequences of feather replacement (Stresseman and Stresseman, '66). The pattern of primary remige molt in cuckoos (Cuculidae) —replacement of the primaries in a series two alternating waves: # 9, 7, 5, 3, 1, 10, 8, 6, 4, and 2—is the most strikingly complex wing molt pattern (Stresseman and Stresseman, '66). The simplest alternative— simultaneous molt of all flight feathers with the temporary loss of flight ability—is a derived condition found only in waterfowl (Anseriformes). The many complex details of molt sequence have evolved by covariation of feather replacement among follicles from various parts of the plumage.

More than annual molt coupled with independence of the plumages grown in different molts can create distinctive plumages in different times of the year that may function in mate choice, intrasexual reproductive communication, or crypsis depending upon the season. Thus, molts can create an entirely different plumage appearance, or distinct alternating plumage modules that are expressed in only one of the annual plumages. The male Ruff (*Philomachus pugnax,* Scolopacidae) has a dramatic change in appearance in its two annual plumages. The alternate (breeding) plumage includes boldly patterned, sexually dimorphic feathers that form a conspicuous ruff around the head, whereas the basic (winter) plumage is composed of entirely cryptically colored, sexually monomorphic feathers of simple shape.

Another effect that can change plumage appearance is feather wear. If the tips of the contour feather barbs are distinctly colored from the base of these barbs, then the gradual wear of the contour feathers can create a dramatic change in plumage appearance without any molt. For example, the male Bobolink (*Dolichonyx oryzivorus*; Icteridae) molts in the winter into a light brownish plumage, but as the tan tips of these feathers wear off, a striking pattern of white rump and wing coverts and black chest is revealed. These contour feathers may be structured for convenient breakage at the point of pigmentation change.

DISCUSSION AND CONCLUSION

A hierarchical perspective on plumage development and evolution provides an important heuristic framework for understanding the diversity and complexity of the plumage phenotype (Figs. 3–6). This conceptual framework can be used to generate new testable hypotheses and can itself be subjected to testing and revision as new data become available.

Recent application of hierarchical modularity to the evolutionary origin of feathers implies that this framework is realistic. Brush ('93, '96) was the first to emphasize the hierarchical nature of feather development, which led him to recognize and emphasize the numerous features that distinguish feathers from plesiomorphic scales. Prum ('99) later proposed a developmental theory of the evolutionary origin of feathers which hypothesized that the causal hierarchy of feather ontogeny could provide specific predictions about the sequence of developmental novelties required for the evolution of complexly branched feathers. The model predicted a transition series of feather morphologies from simple, undifferentiated tubular appendages, to a basally branched tuft of barbs, to a pennaceous vane, and other subsequent structural complexity (Prum, '99).

Recent discoveries of the diversity of feathers in non-avian theropod dinosaurs have confirmed some of the Prum's ('99) predictions about the morphology (Chen et al., '98; Ji et al., '98, 2001; Xu et al., '99a, b, 2000, 2001), and the evolutionary polarity of primitive feathers (Padian, 2001; Sues, 2001; Ji et al., 2001; Xu et al., 2001; Prum and Brush, 2002). Furthermore, a preliminary analysis of distribution of variation in feather morphology

among theropod lineages also provides support to the transition series, or evolutionary order, in primitive feather morphologies (Prum, '99; Prum and Brush, 2002). Support for the predictions of the hierarchical developmental model of the feather origins supports the realism of the hierarchical modularity of feathers that is the basis of the model proposed here. (Also refer to Sawyer and Knapp, Chuong et al., and Homberger in this issue for more discussion on feather evolution.)

More recently, Harris et al. (2002) provided strong molecular developmental support for Prum's model of feather evolution and for a hierarchical conception of plumage complexity. Specifically Harris et al. (2002) document that feather evolution preceded, through the repeated co-option of a plesiomorphic molecular *Shh-Bmp2* module. It appears that the co-option of this plesiomorphic signaling module, with the inherent feature of decoupling of signal function at different times of development, contributed directly to the hierarchical complexity of the plumage phenotype. These advances in our understanding of feather development and evolution were facilitated by a hierarchical conception of the plumage phenotype, and we anticipate that this formalized hierarchical model will provide further opportunities for conceptual and experimental discoveries in this area.

Among the most fundamental insights established by the hierarchical model of the plumage phenotype is the evolutionary relationship between feathers and scales– essentially the boundary between the plumage phenotype and the total of all integumentary appendages. Brush ('93, '96) emphasized the differences between the feather development of feather and scales, and questioned their homology. Prum ('99) emphasized that the most fundamental feather novelty—the tubular feather follicle and feather germ—provided a novel definition of a feather, distinguishing feathers from scales. Harris et al. (2002) document that feathers, bird scales, and crocodilian scales all share detailed molecular homologies at the level of the placode, but that all subsequent stages of the feather development occur through derived, novel mechanisms of morphogenesis. The hierarchical perspective on feather development and evolution has clarified the limited homology of scale and feather placodes, leading to the obvious rejection of numerous traditional, elongate scale-based theories of the origin of feathers (Prum, '99; Prum and Brush, 2002). (Also see Sawyer and Knapp for more discussion on the relationship between feathers and scales.)

There is also genetic evidence for the existence of covariation among plumage modules in birds as hypothesized by the hierarchical model. As mentioned above, the chicken plumage coloration locus known as Columbian Restriction (*Co*) and other similar eumelanin inhibitors limit melanin deposition to feathers of the hackle, wing, tail, and feet (Smyth, '90). Similarly, the White Crested breed of duck has a novel modular tuft of longer feathers on the top of the head. Interestingly, this mutation has no other known phenotypic effects, but it does raise egg mortality to nearly 25% (Metzer Farms, pers. comm.). In both instances, it is known that novel metamodules within the plumage phenotype are determined by single genetic loci. Other domestic breeds, such as Frizzle and Silky chickens (Somes, '90), exhibit plumage phenotypes that are entirely altered by single or few mutations. These mutations with widespread effects indicate the potential of bottom-up effects in keratinogenesis or feather morphogenesis.

The hierarchical model provides an intellectual framework for organizing our current understanding of the plumage phenotype, and for focusing attention on those subjects which deserve attention. Furthermore, the concise hierarchical modularity of avian plumage indicates that avian plumage is an excellent model system for experimental, comparative, and phylogenetic analysis of modularity, hierarchy, and novelty in evolutionary developmental biology. It also raises the compelling question of why feathers are so hierarchically modular? The answer to this question would be an interesting subject of study, but initial solutions can be seen from several directions. As with other integumentary appendages, the iterative repetition of feather follicles over the body provides numerous opportunities for independence and differentiation, but it is really the tubular organization of the epidermal follicle and feather germ—with its inherent capacity for complex nested modular replication, the superimposed spatial polarities within and among modules, and the continuous inductive and nutritional role of the central dermal pulp—that creates the genuinely unique hierarchical complexity of feathers.

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