The origins of species-specific facial morphology: the proof is in the pigeon

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Synopsis One of the principal objectives of developmental research is to understand morphogenesis and in doing so, gain insights into the genetic basis of variation observed throughout the Animal Kingdom. In this review we take an approach, first popularized by Darwin, to understanding how diversity is created by using the domesticated pigeon as a model organism. Nearly 3000 years of selective breeding has produced an astonishing array of feather patterns, behaviors, skeletal shapes, and body sizes. Cumulatively, these features make the pigeon an exemplar of morphological variation. Our research interests center around exploiting the unique properties of domesticated pigeons to gain critical insights into the molecular and cellular basis for craniofacial variation.

Introduction

In 1868, Charles Darwin introduced “The Variation of Animals and Plants under Domestication”, a major work that expanded upon his initial thesis regarding the origin of species. In this work, Darwin’s intention was to provide a sampling of “the amount and nature of changes” he had observed in animals and plants which illustrated the general principles of variation. Darwin wrote, “In one case alone, namely in that of the domestic pigeon, I will describe fully all the chief races, their history, the amount and nature of their differences, and the probable steps by which they have been formed. I have selected this case, because, as we shall hereafter see, the materials are better than in any other; and one case fully described will in fact illustrate all others.” The objective of our research has been to build upon this prodigious work initiated by Darwin.

Almost 3000 years of artificial selection has produced hundreds of distinctive pigeon breeds with unique craniofacial skeletons yet all are derived from a single species, Columba livia. As a consequence, there is an enormous dynamic range of phenotypic variation but it is all contained within one species. Of course, pigeons are not the only animals that exhibit such extreme variations in facial form (dogs and the Galapagos finches come to mind), but none of them offer the immutable advantages of pigeons. In comparison to dogs, these animals have a short gestation period and have minimal requirements for breeding space and become sexually mature within 6 months. These birds breed throughout the spring and summer months and often produce eggs this entire time. Females usually lay two eggs at a time and more frequent laying can be induced by removing the eggs from the nest.

For developmental biologists, birds offer a unique advantage over mammals in that one has ready access to live embryos for experimental manipulation. To those interested in examining the genetic basis for variation, interbreeding among adult pigeons with distinctly different morphologies is feasible as all breeds of pigeons are able to interbreed and produce fertile offspring. Last and most important to the molecular biologist is that molecular and cellular reagents designed for use on chicken embryos work equally well on pigeon embryos. Eggs can be windowed and although we have yet to attempt embryonic injections, we see no reason why pigeon embryos would not be susceptible to a viral (RCAS, adeno, or lenti virus) infection. Further, we are confident that other molecular techniques such as in situ hybridization would be possible in this model system. Thus, in using pigeons one has an availability to impressive armamentarium with which to explore the molecular underpinnings of craniofacial variation.

Analysis of the pigeon genome has been underway for over two decades. A group in Russia published on the organization of sequences in the pigeon genome has been underway for over two decades. A group in Russia published on the organization of sequences in the pigeon genome for Integrative and Comparative Biology, January 3–7, 2007, at Phoenix, Arizona.

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genome and the spectrum of their repetition frequencies in the early 1980s. (Gazarian et al. 1982). More recently pigeon expressed sequence tags (ESTs) have been produced. Primers specific for the ESTs were used to produce amplicons from the genomic DNA pigeons and other economically important avian species whose genome is poorly understood (Smith et al. 2000). Studies such as these are encouraging and serve as proof that the pigeon could soon become a readily utilized model organism.

**Natural versus artificial selection: two sides of the same coin?**

In a larger sense, investigations of this sort provide a window into the mechanisms of evolutionary variation. Consequently, one might legitimately question the use of an animal model whose facial variations are the result of artificial selection rather than natural selection. Darwin described natural selection as a process that “is daily and hourly scrutinizing, throughout the world, the slightest variations... silently and insensibly working, whenever and wherever opportunity offers. We see nothing of these slow changes in progress until the hand of time has marked the lapse of ages...” (Darwin 1859). So is artificial selection analogous to natural selection? The underlying principle of natural selection is that traits which enhance survival and reproduction tend to become more frequent. In artificial selection, the traits, whether they are a dazzling feather pattern or a remarkable beak, are unrelated to survival. In both cases, however, natural and artificial selection act on genes to generate new phenotypes, regardless of whether the process is driven by environmental pressures or a breeder’s fancy. These variations are not unidirectional modifications that creep along on a geological time-scale; rather, they are swift adjustments that inexorably lead towards diversification. Thus, we propose that the pigeon is an ideal model organism in which to gain insights into the developmental mechanisms controlling variation. Our objective is to understand how one trait, craniofacial morphology, is regulated but this unique species is equally adaptable to other studies, as will be seen below.

**Pigeons as a model of morphological variation**

Domesticated pigeons descended from the wild Rock Pigeon and like other domesticates, have been selectively bred for a range of morphological and behavioral characteristics. In particular, the shape of the head and beak has been extensively selected, resulting in pigeon breeds that are at the extremes of distributions of width, length, depth, and curvature (Fig. 1). Qualitatively, this variation easily exceeds that observed in any one wild bird species, and even within that of many bird Families.

The first modern scientific studies of domesticated pigeons were those of Darwin. Indeed, many of Darwin’s key insights into the mechanism of evolution came from his careful observation of variations in domesticated animals. The presence of such large amounts of human-generated variation led him to analogize the nondirected and nonrandom...
action of natural selection with the directed and nonrandom action of artificial selection (Darwin 1859, 1875). Since Darwin, pigeons have been used as a research model in numerous disciplines, and have in many respects become the workhorses of the avian Order. They are easy-going, reliable breeders, and live for up to 20 years as opposed to the very short lifespan of most laboratory animals. Contrary to the derogatory epithet, a bird’s brain is large and complex and pigeons are nothing short of remarkable when it comes to their superlative memory of topographic details. Hence, studies into hippocampal organization have frequently made use of the common pigeon (Kreithen M 1978; Kreithen 1979; Abs 1983; Keeton and Gould 1986; Wallraff et al. 1993; Wallraff 1993, 1994a, 1994b). Pigeons have also been compared to other avian species by the poultry industry (Mondloch 1991, 1995).

The most substantive work on pigeon genetics largely describes breeding schemes and attempts to explain physical variations popular in show breeds in a Mendelian framework. There has been some attempt to place the common pigeon in a phylogenetic context, but only as an overall analysis of Columbiformes (Johnson 2000). There have been limited attempts to systematically characterize skeletal variation in the common pigeon and some domesticated breeds (Johnston 1990, 1992; Johnston and Janiga 1995). Embryological analyses of the pigeon are limited, with most information dating to the early 20th century (Abs 1983). As such, the pigeon represents an underutilized resource in developmental and evolutionary biology. We have begun to develop the pigeon as a model species for studying the developmental mechanisms underlying vertebrate craniofacial variation, but we also envision that this unique animal will be of use in multiple fields of research.

Embryos as evolutionary time capsules

In Ontogeny and Phylogeny Stephen J. Gould (1977) reintroduced the concept that studying embryonic development could shed light on the process of evolutionary change. He suggested that alterations in the rate of growth and in the rate of change, collectively referred to as heterochrony, could lie at the heart of evolutionary variation. In effect, gene mutations that accelerate or decelerate cellular events (e.g., migration, proliferation, differentiation, and apoptosis) adjust how an organ or a tissue develops. There is now strong evidence that even subtle variations in the timing of transcription factor activity may be sufficient to radically alter body plan in both invertebrates (Kim et al. 2000; Esson and Leander 2006) and vertebrates [reviewed by Hall (2003)]. Can the concept of heterochrony be used to explain the craniofacial variations seen in pigeons and other avians? Most assuredly, changes in the rate, duration, and position of cell proliferation could be responsible for the elongated beak of the Scandaroon or the foreshortened beak of the Italian owl pigeon (Fig. 1). But what controls proliferation, differentiation, and apoptosis in this population of postmigratory cells?

**Origin of the cells that populate the face**

The mesenchymal cells that populate the face and form the facial skeleton arise from a subset of epithelial cells that are initially located at the dorsal neural folds. When the neural folds come together during the process of neurulation, cells at the edges of the fold separate from the epithelium and adopt a mesenchymal character. This epithelial to mesenchymal transition marks the birth of cranial neural-crest cells. Many of the molecules involved in the generation, specification, and migration of these cranial neural-crest cells have been identified (reviewed by Helms et al. 2005). Our interest was slightly different: we wondered if cranial neural-crest cells contained information that was required for the species-specific development of the facial skeleton.

As a first step in assessing this question, we exchanged neural-crest cells of the presumptive beak region between quail and duck embryos (Schneider and Helms 2003). This approach exploited three properties that separate ducks and quails: first, quails have beaks that are short, narrow, and convex in comparison to the long, broad, flat bills of ducks. Such distinguishing features offered us a direct way to establish whether facial structures resulting from the transplants more closely resembled the donor or host. Second, quail and duck embryos have considerably different rates of maturation, which meant we could determine the extent to which neural crest regulate gene expression in other tissues involved in facial patterning. Third, quail cells can be detected on the basis of a ubiquitous nuclear marker not present in ducks, which allowed donor and host-derived structures to be distinguished from one another. Neural-crest cells fated to give rise to the beak were then grafted from quail to duck (“quck”) or from duck to quail (“duail”). Chimeric embryos were incubated until St. 28. For controls, we performed grafts within each species, as well as sham operations.
We found that neural-crest cells provide patterning information for beak morphology. Not only do neural-crest cells direct their own morphogenesis, they also pattern tissues not from the neural crest in a manner characteristic of the donor species. Our study revealed that the extent to which beaks are transformed in both size and shape depends on the location and distribution in the duck host of neural-crest cells donated by quail (Schneider and Helms 2003).

Our experiments allowed us to understand the role of neural crest cells in generating inter-specific beak morphology and to determine the extent to which they influence nontransplanted host tissues. Since this study, other groups have studied the molecules involved in craniofacial patterning. Tabin and co-workers evaluated embryos from different species of Galapagos finches and found that alterations in the patterns of Bmp4 expression coincided with differences in beak morphology (Abzhanov et al. 2004; reviewed by Helms and Schneider 2003; Helms et al. 2005). Chuong and colleagues also used inherent differences in avian beak morphology to address whether spatial variations in Bmp4 expression coincided with spatial differences in growth (Wu et al. 2004). We had previously identified an organizing center, the frontonasal ectodermal zone (FEZ), that controls patterning of the middle and upper face region (Hu et al. 2003), and Chuong’s group showed that chicks had a single population of proliferating cells near the FEZ. Ducks, on the other hand, had two such sites situated on the lateral borders of the FEZ (Wu et al. 2004). These areas of cell proliferation coincided with sites of Bmp4 expression in the frontonasal prominence, suggesting that the localized growth zones might be responsible for producing beaks versus bills. When Bmp signaling was overexpressed, or repressed by Noggin, the size of the beaks increased or decreased, respectively (Wu et al. 2004; reviewed by Brugmann et al. 2006). Together, these studies indicate that modulations in Bmp4 activity can alter beak morphology, but they did not clarify whether Bmp4 is instigating these morphological changes or whether its expression is simply changing in response to an upstream mediator.

**Pinpointing the phylotypic and phenotypic stages of facial development in pigeons**

Even a cursory examination of the postnatal pigeon skull reveals dramatic differences in facial skeletal morphology. Darwin speculated that “each successive modification [of these breed-specific facial characteristics], or most of them, may have appeared at an extremely early period...from causes of which we are wholly ignorant.” (Darwin 1875) Our analyses of pigeon embryos bolster this observation made 132 years ago (Fig. 2). Even in pigeon embryos at mid-gestation we could readily detect differences in facial morphology between the Mookie, Common, and Flying flight breeds of pigeons (Fig. 2). Younger avian embryos, however, are indistinguishable from one another, even if they arise from different Orders (pigeons are Columbiformes, whereas chickens are Galliformes; Fig. 2). This stage of embryonic development provide us with a starting point, the phylotypic stage, when breed-specific facial characteristics have not yet developed; and an ending point, the phenotypic stage, when breed-specific facial characteristics are already established. In future studies, we intend to focus on embryonic stages in which breed-specific facial features are identifiable, and use screening tools such as microarray analyses.
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