1 Form and Function

Historical Foundation

The beautiful designs that can be observed in plants and animals have held a fascination for people throughout history. Intimate relationships between form and function inherent in many of these designs are perhaps nowhere as evident as in the musculoskeletal system. In the bones, cartilage, tendons, ligaments, and muscles of all vertebrates there is a gracefully efficient physical order that manifests itself on the organ, tissue, cell, and molecular levels. The existence of such a hierarchy of structural and kinematic harmony is not accidental but the result of unique and complex phylogenetic and ontogenetic histories in which genes and mechanical forces provide critical control. This book addresses the role of mechanical forces in regulating the biological processes that lead to the spatial order, size, shape, and histomorphological characteristics of the skeleton. Throughout this book we refer to this regulatory process as mechanobiology.

The fundamental questions that confront us have been faced by many investigators in the past. In the late eighteenth and early nineteenth centuries, the school of *Naturphilosophie,* championed by Lorenz Oken (Oken, 1809–1811), held that organic order was guided by a divine force that directed the creation of life forms with successively increasing degrees of sophistication and perfection (Gould, 1977). The final level of perfection was thought to be the human form. The Naturphilosophen deemphasized the specific mechanisms of development. The overwhelming consideration was the final organic form itself, and one could be content with the assumption that specific features exist for specific reasons. Those who ascribe to the view that all natural processes move toward a predetermined end are called teleologists or finalists. The function of a structure is, to teleologists, the final argument that explains and justifies the existence of the structure. Giraffes have long necks so that they can eat the leaves in tall trees. Monkeys have tails so that they can better swing through trees. The shafts of most long bones are hollow in order to provide a place for marrow.

Naturphilosophie had as its foundation the ideas of the ancient philosophers, and its tenets were reinforced by the religious and moral climate of the times. Aristotle said "Nature does nothing in vain," and the strong belief in God's role in creating that natural order was a fundamental Christian principle (Mayr, 1991). In the nineteenth century, William Paley's widely studied text, *Natural Theology,* argued that the intricate designs of living creatures provide a convincing argument for the existence of God (Paley, 1802). This doctrine of "argument by design" proposed that the mere existence of such marvelous order offered overwhelming evidence for the existence of a Creator. The attribution of structural features of organisms to their "designed" function appealed to human experience, and the teleologic approach to morphology was easily accepted.

Alfred Russel Wallace and Charles Darwin began to alter this view of the structure and order of life when they introduced the concept of natural selection. In *The Origin of Species,* first published in 1859, Darwin viewed the evolution of different life forms as a long series of minor heritable changes (Darwin, 1872). The survival and propagation of an organism was hypothesized to be dependent on the principle of the survival of the fittest. Random variations cause alterations in developed forms. Variations leading to the development of beneficial features increase the liklihood that the animal will survive into sexual maturity. Whereas other variations lead to features that reduce fitness, thus producing a greater tendency for extinction. The increasing sophistication and capabilities evident at higher phylogenetic levels were therefore hypothesized to result from processes of variation and elimination. Darwin rejected teleology as well as the belief that the features of organisms necessarily achieve perfection through evolution.

Physical adaptation of species to the environment was of considerable interest to Darwin. He speculated about a direct effect of the environment on certain structures and hypothesized that environmental influences could increase variability. Furthermore, in conjunction with his theory of natural selection, Darwin continued to accept the Lamarckian principle (Lamarck, 1809) of the heritability of acquired traits. According to this tenet, the use or disuse of organs and tissues would cause physical adaptations, and, over many generations, these adaptations would gradually appear in offspring. Darwin therefore stopped short of attributing all evolutionary change to purely stochastic processes of variability and selection.

The rejection of the principle of acquired characteristics began in 1883 with August Weismann who denied the effects of use and disuse in evolution (Weismann, 1883). He felt that a clear distinction in evolution theory should be made between the transmutation theory and the theory of natural selection (Mayr, 1991). Weismann also recognized that the range of characteristics that can appear in evolution is constrained by the biological processes involved in development (Mayr, 1991). This important observation has received increasing attention in recent years. Although many organisms and various histomorphological characteristics can be imagined, relatively few can be formed through the biological processes of normal development. The restrictions on biological form as a result of the developmental process are referred to as "developmental constraints."

Developmental constraints contribute to "evolutionary constraints." The evolutionary forms that are possible, therefore, comprise only a small subset of the forms that are imaginable.

The full demise of the theory of acquired characteristics was not achieved until the mid-twentieth century with discovery of the genetic code by James Watson and Francis Crick (Watson and Crick, 1953a, 1953b) and the subsequent rapid advances in molecular biology. There is simply no mechanism by which traits acquired through use or disuse can directly alter the order of DNA base pairs in the genes that are passed to the offspring. The rejection of the principle of acquired characteristics from Darwin's initial evolutionary perspective led to what has been referred to as neo-Darwinism, which forms the basis of modern evolutionism.

The rejection of the theory of acquired characteristics makes it difficult to account for the appearance of many traits that begin to appear in the embryo and yet play an important functional role only after birth. Weismann and others have proposed that all reputed cases of such inheritance could be adequately explained by random variations and direct natural selection. Conrad Waddington, however, argued that it is unlikely that chance mutations lead to the features in newborns that have immediate functional value (Waddington, 1975). As one example, he refers to callosities of the ostrich, which appear in the embryo but are undoubtedly related to the crouching posture of the bird after hatching. These callosities are analogous to thickening of the soles of the feet in human embryos that Darwin felt was an acquired characteristic that derived from the thickening of adult soles in response to the mechanical stresses imposed by walking (Darwin, 1871).

To account for these types of features, Waddington advanced the notion of genetic assimilation. This idea incorporates the observation that cells respond to use and disuse so as to alter some trait in the individual. Although the adapted trait itself is not heritable, the response sensitivity and magnitude are genetically determined and subject to variation. Some individuals may have a particularly sensitive and strong response, leading to the appearance of very beneficial traits (such as thicker skin on the soles of the feet). Those individuals will then be selected, and the sensitivity and magnitude of the response will be inherited by the offspring. In this way, the response itself could become more sensitive and stronger over many generations. Eventually, the trait becomes *canalized* to the extent that it appears with minimal stimulation and can arise in development even before the function begins.

Despite the general acceptance of selection theory and the clear dependence of evolution on the mechanisms of development, there is still a strong sentiment to retain elements of teleological reasoning in viewing morphology. An argument in support of the teleological view is based on the assumption that genetic variations over hundreds of millions of years have been so numerous that an almost infinite array of variations have occurred. The cumulative result of natural selection, some have suggested, is the progressive attainment of the best or "optimum" solution. Many implicitly assume that the mere existence of specific skeletal features suggests

that these features fulfill some hypothetical design goal that is inherent in the process of natural selection itself.

Teleological reasoning, which is still pervasive in the life sciences, has the potential of misleading the search for morphogenetic and evolutionary causation. Many features of animals may simply be artifacts of underlying developmental processes and provide no particular advantage or disadvantage to the organism (Gould and Lewontin, 1979). Other features may be a simple consequence of fundamental physical laws such as gravitational forces or thermodynamic principles (O'Grady, 1984; Thompson, 1992). Julius Wolff, after his extensive observations on skeletal adaptations, concluded in 1892 that a teleological approach to understanding the shape and structure of bones was untenable (Wolff, 1986). Many morphological features in the skeleton appear due to direct biological responses to mechanical stimuli. In his classic text *Das Gesetz der Transformation der Knochen* (*The Law of Bone Remodelling,* English translation by Maquet and Furlong, 1986), Wolff wrote:

> Darwin and Wallace related the development of appropriate arrangements in the organisms only to selection among morphological variations in the struggle for life among the individuals. However, they left a gap in their explanations. They did not explain the self-shaping of the appropriate structures inside the organs of the living bodies, neither in normal nor abnormal circumstances. (Wolff, p. 118)

Wolff's ideas on the causal relationship between physical forces and morphological modifications during life were in concert with the views of Wilhelm Roux and Emil du Bois-Reymond. Wolff considered the "functional adaptation" of the individual in relationship to selection theory and argued that the design of an organism for its function is a result of both influences. He wrote:

> Roux, as I do myself, distinguishes two periods in the life of every organism. One is embryonic. During this period "the organs expand, differentiate and grow." The other period is adulthood. During this period growth and replacement of what is worn out take place "only when stimulated." "The stimuli can also produce new structures which, when forced to appear during several generations, become hereditary. They then appear in the embryo without further need for these stimuli." Embryonic life ends and "stimulated life" begins probably at different times for each tissue and for each organ. As a rule, these organs which perform their function already in the embryo will have a stimulus life even in the embryo, depending on the degree of function. Roux explains the "stimulated life" in embryonic as well as in pathological conditions by his hypothesis of the "trophic action of the functional stimuli" and by the consequent principle of the "direct functional self-shaping of the appropriate structure." (Wolff, p. 72)

Wolff's statement presents two concepts of particular note. The first is the Lamarckian tenet on the heritability of acquired traits, which was supported by Darwin, Wolff, Roux, and most other biologists of the time. The second concept is Roux's belief that physical forces, at some point in development, begin to influence the morphogenetic processes. Roux (1895) referred to the collective physiochemi-

cal processes in development as *Entwicklungsmechanik,* or "developmental mechanics." As will be demonstrated, the appreciation of developmental mechanics in the skeletal system may lead to a better understanding of the interrelationships between physical function and development, aging, and evolution. Furthermore, we believe that the biophysical principles embodied by developmental mechanics are intimately involved with histomorphological alterations, tissue regeneration, tissue differentiation, and the pathogenesis of some skeletal diseases. The viewpoint of the present book resonates with ideas expressed by previous investigators, including Galileo (Galilei, 1939), Roux (Roux, 1895), Wolff (Wolff, 1986), Thompson (Thompson, 1992), and Pauwels (Pauwels, 1980).

Skeletal Pattern Formation

Early preformationists believed that the characteristic features of an animal were fully formed in miniature within a gamete at the time of conception (Figure 1.1). Simple growth of this miniature organism was believed to be the mechanism of maturation. However, with scientific advancement, opinions began to change. By the eighteenth century it was generally conceded that conception was merely the initial stage of the long, complicated process of growth and development. Karl von Baer (1792–1876) and others argued that conception resulted in the assembly of the necessary biological components within the gamete. These components provided the capability for the progressive development of features characteristic of a specific organism. This theory of development was referred to as "epigenesis" and

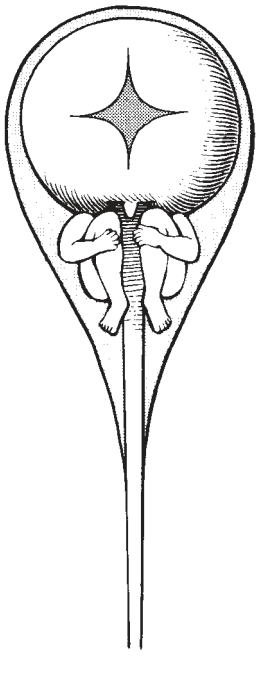

Figure 1.1. Seventeenth-century preformationists' view of a miniature person within the human sperm. Copy of a drawing by Hartsoeker (from Moore, 1982).

stood in stark contrast to the preformationist approach (Gould, 1977). It is now well known that the initial fertilization of the ovum results in the creation of a single cell with the necessary genetic information for organismal development. What follows is an extremely complicated orchestration of cell division, differentiation, growth, and organization by which the characteristic features of an organism are progressively developed.

Fertilization begins as the sperm enters the ovum and is complete when the maternal and paternal chromosomes combine during the first mitotic division of the resulting zygote. Rapid cell division then leads to the development of a cell mass, the blastocyst, which attaches to the wall of the uterus. The inner cell mass of the blastocyst comprises the embryoblast, from which the embryo forms.

By the end of the third week, rapid cell proliferation and differentiation have converted the blastocyst to a structure referred to as the trilaminar embryonic disc. The embryo at this stage consists of three germ layers: the embryonic ectoderm, mesoderm, and endoderm. It is from these three fundamental germ layers that all of the tissues and organs of the body are derived (Moore, 1982).

The mesoderm of the young embryo contains only spindle or star-shaped cells called mesenchymal cells. Additional mesenchymal cells also arise from the adjacent ectoderm in the cephalic region of the neural crest and neural tube. Mesenchymal cells are the most pluripotential cells in the body. The proliferation, migration, aggregation, and differentiation of mesenchyme during subsequent embryonic development lead to the formation of many different tissue types and organs, including the connective tissue and muscles of the viscera, trunk and limbs, the cardiovascular and lymphatic systems, blood and lymph cells, the dermis of skin, and dentine. Furthermore, the entire skeletal system and associated tissues, including cartilage, bones, tendons, and ligaments, are derived from pluripotential embryonic mesenchyme.

In the course of animal development, cell division increases the number of cells from one to 10^{11}. In addition, different cells, although they all contain the same genetic information, begin to selectively express different genes. By these processes of selective gene expression, the cells differentiate into approximately 200 different cell types that are organized in elaborate patterns with characteristic extracellular matrix products (Edelman, 1988). Establishing the genetic and physicochemical mechanisms that regulate the generation of patterns of differentiated tissues in development represents a major challenge in developmental biology (Melton, 1991).

The basic framework or "Bauplan" of the vertebrate skeleton has been consistent across taxonomic groups for approximately 500 million years (Gould, 1989). The organization of the tetrapod limb has not changed since the Devonian period. It consists of a single long bone (humerus, femur) which articulates with the body, followed by two parallel bones (radius/ulna, tibia/fibula) and an array of hand and foot bones (Shubin, Tabin, and Carroll, 1997). Most recent attempts to understand the evolution of axial and appendicular skeleton have followed the approach of developmental genetics (Kingsley, 1994; Burke, Nelson, et al., 1995; Storm and

Kingsley, 1996; Marker, Seung, et al., 1997; Shubin, Tabin, and Carroll, 1997). Modifications in skeletal organization have been correlated with regulatory changes in specific patterning genes. Many of these genes are highly conserved in evolution, and therefore similar genes appear in very different animals, including vertebrates and arthropods (e.g., insects, arachnids, and crustaceans).

Hox genes represent a subclass of homeobox selector genes that are crucially involved in initial pattern formation in the axial skeleton and play a key role in limb morphogenesis (Burke, Nelson et al., 1995; Kappen, 1996; Shubin, Tabin, and Carroll, 1997; Upholt, 1998). Many other genes including several in the TGF-β superfamily (including BMPs and GDFs) and the Wnt and hedgehog families have been implicated in skeletal patterning (Kingsley 1994; Storm and Kingsley, 1996; Shubin, Tabin, and Carroll, 1997; Tuan 1998). Once the skeletal pattern is formed, the expression of some of these genes continues to influence skeletal growth and differentiation. HoxC-8 and Indian hedgehog (Ihh) have been shown to regulate cartilage growth in the skeleton (Vortkamp, Lee, et al., 1996; Kappen, 1998). The CBFA-1 gene has been identified as a crucial transcription factor for bone formation since it causes pre-osteoblasts to become osteoblasts and start producing osteocalcin (Dickman, 1997). There has been an explosion of research on peptide signaling molecules called growth factors, differentiation factors, cytokines, and their receptors that are thought to be downstream targets of regulatory genes. Some factors that have been demonstrated to influence skeletal tissue biology include parathyroid hormone related protein (PTHrp), insulin-like growth factor (IGF), fibroblast growth factors (FGFs), transforming growth factor-beta (TGF-β), cartilage-derived growth factors (CGFs), cartilage-inducing factors (CIFs), bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs), and interlukins (ILs). Mechanical stimuli may regulate the expression, synthesis, and degradation of some of these factors and their receptors, as well as many other genes.

The genes of the fertilized ovum do not contain the "blueprints" for direct formation of specific histological and morphological features of an organism. Development is an extremely complex process that proceeds by an elaborate orchestration of gene expression over time and space that is regulated by some of the genes themselves (regulatory genes) as well as by interactive cell and matrix mechanochemical events. These two modes of regulating developmental processes are sometimes referred to as genetic (or intrinsic) and epigenetic (extrinsic) control. The modern use of the word "epigenesis" has thus changed from its original application in that the term now applies to the regulation of gene expression and cell biology by environmental, physical, and chemical factors.

One of the most important factors in the regulation of gene expression, cell metabolism, and matrix synthesis during embryogenesis is the interaction among developing tissues. These interactions are epigenetic events that may take the form of direct cell-to-cell or cell-matrix contact, cell-cell or cell-matrix physical forces, or the production of diffusive molecules, called morphogens, which can influence cell function. In addition to the genes and growth factors mentioned above, retinoic acid, hyaluronate, calcium, and oxygen have been implicated as mor-

phogens involved in embryonic skeletogenesis. Epigenetic chemical factors play a major role in the progressive attainment of pattern and shape in developing organisms and have a profound influence on cartilage tissue development (Urist, DeLange, and Finermen, 1983).

The ongoing identification of regulatory genes and morphogens is extremely important to understanding development but does not, by itself, explain how different biological patterns are actually generated (Horder, 1993). Early attempts to show how genetic and epigenetic factors may regulate pattern formation and morphogenesis used mathematical models that emulate the cumulative effect of many spatially varying cellular events over some period of time. The first mathematical simulations involved chemical prepattern models derived from the reaction-diffusion hypothesis of Turing (Turing, 1952). These models postulated that steady-state distributions of morphogens are created within developing tissues. Morphogen concentrations could locally influence such processes as cell death, migration, differentiation, proliferation, or assembly. Different locations within the tissue could serve as "sources" or "sinks" for these morphogens (Crick, 1970). Differential equations for autocatalysis, decay, and diffusion can then be used to describe the spatial and temporal distributions of the morphogens. The solutions of these equations provide the mathematical description of the complicated patterns that could arise in the tissues.

A related form of the chemical prepattern models was incorporated in the "positional information" ideas of Lewis Wolpert (Wolpert, 1978). Positional information concepts are based on the premise that every cell within a developing tissue region is somehow endowed with a positional value with respect to adjacent cells. That value is then utilized to direct the expression of specific genetic information.

Research on the embryonic pattern formation of skeletal rudiments has often focused on the developing limbs. At approximately 4 weeks, the aggregation of mesenchymal cells forms masses that constitute the embryonic limb buds. Growth and differentiation of these cells lead to the morphogenetic patterns of tissues within the upper and lower limbs (Figure 1.2). Regulatory genes, tissue interactions, diffusible morphogens, and tissue tractions associated with growth and osmotic forces all play critical roles in limb development.

Perhaps the most rigorous mathematical attempts to describe development of the early cartilaginous skeletal elements were the mechanochemical models of Oster, Murray, and associates (Oster, Murray, and Maini, 1985; Oster, Shubin, et al., 1988). They pointed out that complete reliance on a simple spatial distribution of morphogens created in a preestablished field ignores the true sequential developmental stages of embryonic growth and development in the limbs. Cell division, migration, differentiation, extracellular matrix production, and tissue stresses are rapidly changing events that are strongly influenced by immediately preceding states. They feel that mechanical and osmotic forces created during embryonic development may be crucial in forming the tissue patterns that emerge during this rapid period of growth. The importance of cell-cell and cell-matrix forces during development is widely recognized by others who have stressed the importance of

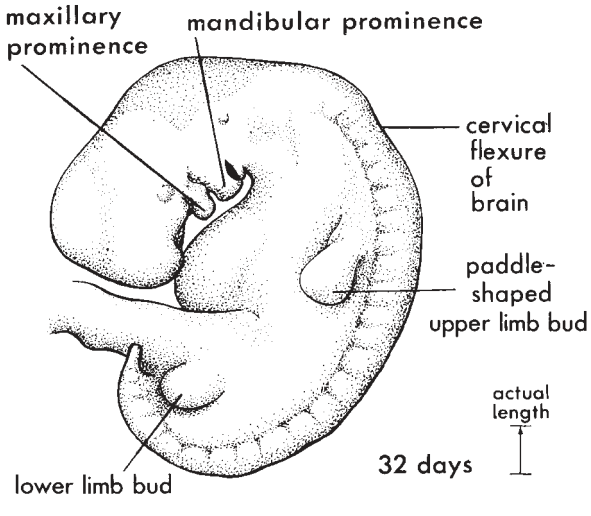

Figure 1.2. The human embryo during the fifth week. Subsequent limb bud growth and differentiation form the appendicular skeleton (from Moore, 1982).

cell adhesion molecules (CAMs) and surface adhesion molecules (SAMs) in embryonic development (Edelman, 1988).

The differences and similarities of chemical prepattern and mechanochemical models for embryonic development of basic skeletal elements of the limbs are reviewed by Oster et al. (Oster, Shubin, et al., 1988). In the course of limb bud growth, a central feature in both classes of models is the instabilities in the solutions of the nonlinear differential equations that represent chemical concentrations and reactions, and mechanical and osmotic forces. These instabilities lead to segmentation and bifurcation of the chondrogenic condensations (Figure 1.3).

These mathematically predicted bifurcations can simulate the physical bifurcations observed experimentally (Figure 1.4).

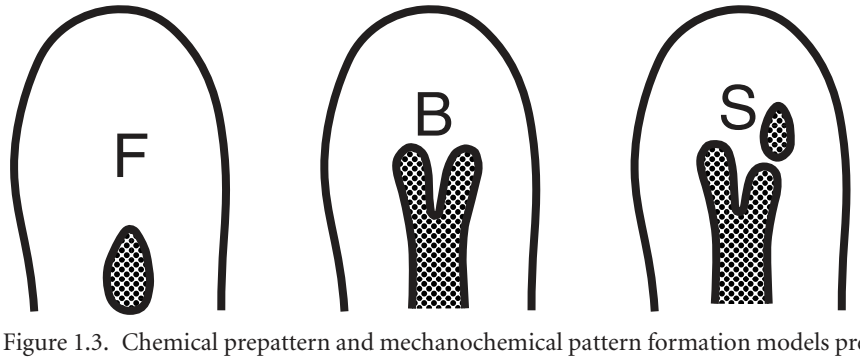

Figure 1.3. Chemical prepattern and mechanochemical pattern formation models predict three possible types of cartilage condensations: (left) focal condensations, *F;* (center) branching bifurcations, *B;* (right) segmental bifurcations, *S* (adapted from Oster, Shubin, et al., 1988).

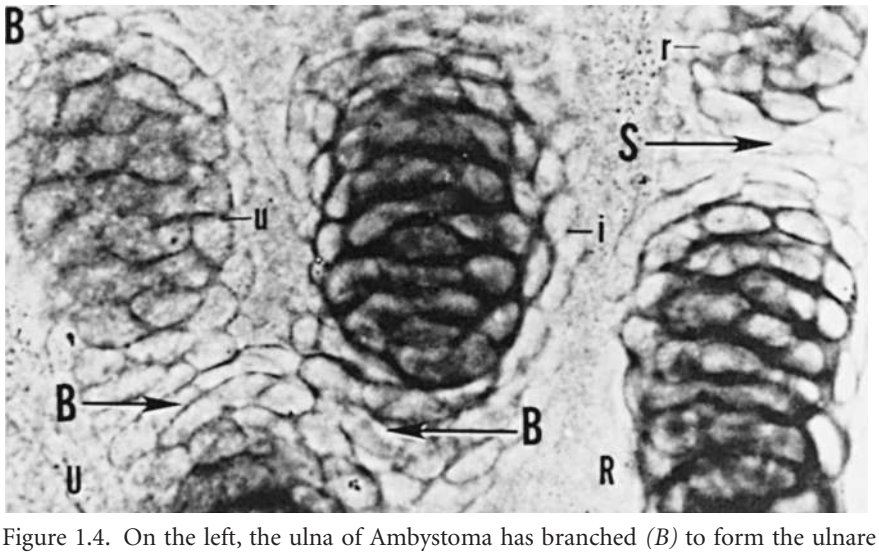

Figure 1.4. On the left, the ulna of Ambystoma has branched *(B)* to form the ulnare *(u)* and intermedium *(i)*. This has displaced the segmentation *(S)* of the radius *(R)* from the radiale *(r)* distally (from Shubin and Alberch, 1986).

Local tissue geometry, cell mitosis and migration rates, matrix synthesis rates, chemical gradients, and interactions with adjacent tissue may play a role in determining if and when condensations and bifurcations occur. Furthermore, recent work seems to indicate that the expression of GDFs and possibly other genes are intimately associated with presumptive sites of joint formation (Kingsley, 1998). The net result of the sequences of condensation, segmentation, and bifurcation during limb bud growth is a distinctive pattern of cartilaginous elements that are the anlagen or "templates" of the future limb bones. Further skeletal growth and development are achieved by the growth and ossification of these rudiments.

When the mechanisms of development are appreciated, it becomes clear that totally random developmental variations cannot occur. Rather, any variations in morphology must be consistent with the molecular genetic and mechanobiological "morphogenetic rules" that guide the developmental process. These factors define developmental constraints on the type of limb bone organizations that can appear in the evolution of vertebrates (Figure 1.5). An argument based on molecular genetics can be made to explain why in the evolution of early tetrapods, digits appeared simultaneously in the forelimbs and hindlimbs. No Devonian tetrapod had fingers but not toes (Shubin, Tabin, and Carroll, 1997). The mechanobiological basis of the appearance of many skeletal features also presents developmental constraints on the general skeletal features that appear in particular taxa. For example, since mechanical stimuli regulate the development of the cross-sectional shape of long bones (van der Meulen and Carter, 1995), random variations in bone shaft geometry cannot occur. Similarly, since cartilage growth and ossification is regulated by mechanical stresses (Carter, Orr, et al., 1987; Wong