For centuries, biologists have been intrigued by three fundamental questions: (1) Why do all organisms persistently attain size-correlated variations in their form and processes? (2) What causes the morphological, anatomical, and physiological changes of organisms in the progression from fertilized ovum to mature form? (3) How do individual organisms adjust themselves to adapt well to the varying environments in which they are reared? In the past, these questions have been addressed through the concepts of allometry (Huxley 1924), ontogeny (Gould 1966), and phenotypic plasticity (Woltereck 1909). Allometry describes the size of a part of an organism as a proportion of the whole. The ontogeny of an organism includes the entire sequence of events occurring from the single-cell through the adult stages. Phenotypic plasticity refers to multiple phenotypes of a given genotype produced in an array of environments. Differences in biological traits affecting allometric scaling, ontogenetic change, and environment-stimulated plasticity have been uniquely powerful in their ability to explain systematic and predictable scale dependencies in the anatomy and physiology of organisms.

Gould and Lloyd (1999) argued that the concepts of allometry and ontogeny could be integrated across three hierarchical levels: the gene, the organism, and the species. Data about the two higher levels have accumulated at a substantial rate in the past decades, but information at the gene level has remained sparse. Modern molecular marker technologies, in conjunction with powerful statistical methods, provide a powerful means for identifying and mapping genetic loci that contribute to quantitatively inherited traits (termed quantitative trait locus [loci], or QTL) in organisms (Lander and Botstein 1989, Lark et al. 1995). More recently, we have developed a general framework for genetic mapping of the QTL responsible for the allometric relationships between different traits (Wu et al. 2002a, Ma et al. 2003) and for variation in size and shape during ontogeny (Ma et al. 2002, Wu et al. 2002b). From a statistical perspective, our approach can significantly increase the precision and power of the estimation of QTL parameters, because the implementation of biological principles leads to a reduction in the number of unknown parameters being estimated. Biologically, our approach gives mechanistic insight into how the allometry, ontogeny, and plasticity producing the vast diversity of living organisms are modified by genetic changes.

In this article, we first review the developmental features of allometry, ontogeny, and plasticity and show how these three features of an organism at the interface between development and evolution, researchers must understand their underlying genetic bases. We have developed a general framework for deciphering the genetic machinery that guides allometric scaling, ontogenetic growth, and environment-dependent plasticity in biological organisms. This approach constitutes a step toward creating a unified view of evolutionary biology and developmental biology (“evo-devo”).

Keywords: allometry, developmental biology, genome, ontogeny, plasticity
concepts are described by mathematical models. We then introduce a statistical mapping framework, which builds on the mathematical models, and describe how it can be used for the molecular dissection of allometry, ontogeny, and plasticity. Finally, we suggest that a genome-mapping approach should be employed to integrate the knowledge about evolutionary and developmental biology obtained at different levels of organizational complexity.

**Allometric scaling of organisms**

The diagram in figure 1 strikingly illustrates the changes in the form and proportion of the human body during the fetal and postnatal stages. There are remarkable developmental changes in the size of the head and limbs in relation to the size of the body. For example, the head is about one-fourth of the newborn infant’s body but only about one-twelfth of the adult’s. The trunk is a much larger portion of the infant’s body than the adult’s, while the leg of the adult is proportionately twice as long as the newborn’s. All human beings share the same mechanisms of growth and development and proceed through the same stages of life.

The proportional relationship of the size of organs to the size of the whole body, a field termed **allometry** by J. S. Huxley 77 years ago, is widely used to characterize overarching patterns and constraints in animal biology. Recently, comparisons of plants with animals have been made in terms of the relations between size and form, and between metabolism and reproductive effort, and the potential causes of these relations (Niklas 1994, Reich 2001). Allometry can be defined in four different ways (Cheverud 1982): **ontogenetic allometry**, the growth trajectory of an organ relative to body size during the growth of a single individual; **static allometry**, the scaling relationship among individuals between one organ and total body size at a single developmental stage; **plastic allometry**, the size relationship between organs across environments; and **evolutionary allometry**, the size relationship between organs across species. Although they are similar to each other, these four types of allometry often reveal different developmental aspects of organisms’ size and shape control.

Allometry is a biological scaling of form and process, and it can be defined mathematically. If $y$ is a variable—for example, body length or metabolic rate—and $x$ is the body mass for an individual, the allometric relationship of $x$ and $y$ obeys the exponential model $y = y_0 x^k$, where $y_0$ is a normalization constant and $k$ is a scaling power. Although the constant $y_0$ changes across species, age, and environment in which the organism is reared, it is thought that the power $k$ only changes depending on the specific organ–whole organism relationship under study, regardless of species, age, and environment (Niklas and Enquist 2001). For example, population density always scales as approximately the $-3/4$ power of body mass, whereas metabolic rate always scales as approximately the $3/4$ power of body mass, regardless of species. The allometric scaling between growth rate and mass can be shown at the cellular level (figure 2). The power is 0.76 to 0.78, which is close to the expected value of $3/4$. In biology, there are many (about 200) fixed scaling powers in which the denominator is a multiple of four (Whitfield 2001).

Why does an organism scale as a fixed power of its body mass? If we examine the relationships between the length, surface area, and mass of a geometric object, we see that the length scales as the $1/3$ power of mass and the area as the $2/3$ power. Because metabolic rate is linearly related to surface area, metabolic rate should also scale as the $2/3$ power of mass. However, these expectations are not consistent with the $3/4$-power relationship observed. West and colleagues (1997, 1999) have recently developed the fractal scaling theory to explain this discrepancy. If an organism is viewed as a fractal-like network system for the absorption and internal distribution of metabolites, one can see that the length scales as the $1/4$ (not $1/3$) power of body mass and the area as the $3/4$ (not $2/3$) power of body mass.

**Ontogenetic trajectories**

Allometry is a function of development. The developmental process is described by the concept of ontogeny (Gould

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**Figure 1. Changes in human body proportion from the second fetal month to adulthood. Adapted from Robbins (1928).**
For more than half a century, genetics and natural selection have been regarded as two pillars in the study of evolution. However, theorists often view adults as the final destination of evolution and have omitted what selection acts upon—ontogeny. Ontogenies evolve, as do adults. Mutated genes are passed on only to the extent that they promote survival of ontogenies, and adulthood is only a fraction of ontogeny.

Different developmental events and their consequences can occur throughout ontogenetic trajectories, in which morphological or physiological characteristics are expressed as a function of time \( t \) from the earliest stages of embryogenesis to maturity (Schlichting and Pigliucci 1998). For example, it is possible that the remarkable sex-specific variation in the size of adult humans results from differences in timing in the onset of sexual maturity. Morphological changes also occur in leaf size and shape during ontogeny for wild and cultivated cucubit plants (figure 3). The developmental changes from very young leaves to very old leaves in this annual plant are dramatic. Overall, leaf sizes are larger for the cultivated than for the wild types, because humans have selected these plants for yield, which is strongly associated with leaf area.

The difference in leaf ontogenetic trajectories between wild and cultivated cucubits (figure 3) represents an example of heterochrony, the change of a characteristic from the time of its appearance in an ancestor to its appearance in a descendant. Change in relative timing can occur at any time during ontogeny, that is, any time between fertilization and maturity. Heterochrony is a mechanistic embodiment of the relationship between ontogeny and phylogeny (Alberch et al. 1979). It is the effect of alterations to the timing and rate of ontogenetic development and, as such, is one of the cornerstones of evolution.

### Growth models

The evolution of ontogenetic trajectory can be studied by formulating the underlying growth laws using a mathematical model (Alberch et al. 1979). A growth law can be visualized as the "force field" propelling a point through a phenotypic space, tracing out the ontogenetic path. If the size of an organism is denoted by \( y \), its ontogenetic trajectory, \( y(t) \), can be generated through the differential \( dy/dt \), which models the growth rate. The establishment of a universal growth law is usually based on empirical goodness of fit to the data (Alberch et al. 1979). Recently, West and colleagues (2001) derived a general model for ontogenetic growth based on fundamental principles for the allocation of metabolic energy between maintenance of existing tissue and production of new biomass.

According to Alberch and colleagues (1979), there are three major established categories of growth models: exponential, saturating, and sigmoidal. For each of these growth models, the development of the ontogenetic trajectory is regulated by a set of “control parameters” such as onset age of growth, offset signal for growth, growth rate during the period of growth, and initial size at the commencement of the growth period. Each model exhibits an initial phase of exponential growth caused by the geometrically multiplying population of newly differentiated cells. Because of this exponential growth, small perturbations in rate or age of onset are amplified enormously during ontogeny. Thus, there are many examples of small changes in a growth parameter that cause a series of developmental alterations and produce a phenotype qualitatively different from the normal one (Alberch et al. 1979).

### Phenotypic plasticity: A reaction norm model

The concept of phenotypic plasticity has been popular in evolutionary biology in the last two decades. Phenotypic plasticity can be defined as the capacity of a genotype to produce different phenotypes when exposed to different environments. Plasticity is therefore a property of the reaction norm of a genotype, which is the function relating...
environmental input to phenotypic output through certain genetic machinery (Schlichting and Pigliucci 1998). Phenotypic plasticity can be observed at the morphological, developmental, anatomical, physiological, and biochemical levels. An excellent example of phenotypic plasticity is illustrated in figure 4, using butterflies as a model system. The adult female satyrine butterfly develops larger forewing eyespots in the wet season than in the dry season (Brakefield et al. 1996). Such a phenotypic divergence (figure 4, z-axis) is a consequence of the adaptation of the insect to a particular environmental variable (water availability in this example; figure 4, x-axis). This environment-stimulated divergence increases from the fifth instar to pupation to adulthood (figure 4, y-axis), indicating that phenotypic plasticity interacts with development to control forewing eyespot.

Plastic responses to the environment have four attributes: amount, pattern, rapidity, and reversibility (Schlichting and Pigliucci 1998). The amount of plasticity describes the magnitude of response to the environmental change; the pattern refers to the shape of response; the rapidity is the speed of response characterized by the slope of a reaction norm curve; and the reversibility refers to the capacity for switching between alternative states. The concept of phenotypic plasticity should embody these attributes. Considerable attempts have been made to investigate what developmental and physiological mechanisms are modulated to yield phenotypic variation in divergent environments (Schlichting and Pigliucci 1998).

The genetic study of phenotypic plasticity has produced controversy. How do the genes affecting the plastic response operate? Indeed, do genes for plasticity really exist? Presumably, there are two fundamental categories of genetic control over plasticity: allelic sensitivity and regulatory plastic genes (Schlichting and Pigliucci 1998). The first one causes phenotypic modulation, a developmental phenomenon, in which the phenotypic outcome is a continuous function of the environmental change. The second one corresponds to developmental conversion, which is characterized by the production of distinct morphs separated by thresholds. Brakefield and colleagues (1996) found that the larger eyespots of butterflies that develop in the wet season are associated with stronger expression of the gene Distal-less, whereas the smaller eyespots that develop in the dry season are associated with weaker expression of this gene (figure 4). However, the expression of Distal-less is also subject to developmental control; for example, it is silent until the butterflies pupate. Precise genetic control mechanisms that affect phenotypic plasticity are believed to be fundamentally important in the evolution of phenotypes that are expressed in different environments.

A conceptual mapping framework of developmental biology
What intrinsic factors operate to provide the wide variety of phenotypes on which natural selection can work? Researchers are bridging the gap between phenotypes and natural selection by placing mutations of adaptive significance within a structured framework, thereby contributing toward a more cohesive theory of evolution and improving understanding of how allometries, ontogenies, and plasticities evolve. Quantitative trait loci governing scaling relationships, ontogenetic growth, and phenotypic plasticity can be mapped using polymorphic markers. This provides a key step toward understanding the structure and function of the genes responsible for these developmental features (Rougvie 2001). Although the genetic mapping of these three features can be viewed as a multivariate trait problem, current statistical models for mapping multiple traits are limited because the biological mechanisms behind allometry, ontogeny, and plasticity are not considered. By integrating these pervasive scaling laws, growth

Figure 3. Developmental differences in leaf shape between wild Cucurbita argyrosperma sororia (left) and cultivated Cucurbita argyrosperma argyrosperma (right). Notice the difference in scale. Numbers are denoted as a surrogate for the time of leaf development. Adapted from Schlichting and Pigliucci (1998).
laws, and reaction norm laws into statistical models for QTL analysis, we have developed a general framework for mapping the QTL that affect allometry, ontogeny, and plasticity (Wu et al. 2002a, 2002b, Ma et al. 2002, 2003). This approach allows for tests of whether the allometric relationship of two traits is affected by a single QTL or by more than one linked QTL. Also, under this model, a number of biological hypotheses related to the interface of evolution and development—evolutionary developmental biology, or “evo-devo” (Raff 2000, Arthur 2002, Beldade and Brakefield 2002)—become testable.

The basic statistical model for mapping QTL is a mixture model, in which each observation \( y \) is assumed to have arisen from one of \( m \) groups of QTL genotypes, each group being modeled by a density from the parametric family \( f \). The population density function of \( y \) is

\[
p(y|\pi, \phi, \eta) = \pi_1 f(y; \phi_1, \eta) + \ldots + \pi_m f(y; \phi_m, \eta),
\]

where \( \pi \) represents the mixture proportions \((\pi_1, \ldots, \pi_m)\), which are constrained to be nonnegative and sum to unity; \( \phi \) represents the genotype-specific parameters \((\phi_1, \ldots, \phi_m)\), with \( \phi_j \) being specific to genotype group \( j \); and \( \eta \) is a parameter common to all genotype groups. The mixture proportions denoted as the frequencies of QTL genotypes depend on the marker genotypes of two flanking markers bracketing the QTL. The normal density functions associated with different QTL genotypes are expressed in terms of the expected value—evolutionary developmental biology, or “evo-devo” (Raff 2000, Arthur 2002, Beldade and Brakefield 2002)—become testable.

The normal density functions associated with different QTL genotypes are expressed in terms of the expected value of each genotype.

For QTL mapping of allometry, ontogeny, and plasticity, we measured multivariate phenotypes for each individual (denoted by a vector \( y \)) on multiple traits, or on a single trait at many different time points or under different environmental regimes. Thus, the phenotypes of each QTL genotype group follow a multivariate normal density,

\[
f_j(y) = \frac{1}{(2\pi)^{m/2}|\Sigma|^{1/2}} \exp\left(-\frac{(y - u_j)^T \Sigma^{-1} (y - u_j)/2}{2}\right),
\]

where \( T \) denotes the transpose operator, \( u_j \) is the vector of the expected genotypic values of QTL genotype \( j \) for all \( m \) variables, and \( \Sigma \) is the \((m \times m)\) residual variance–covariance matrix of the variables. Indeed, \( u_j \) can be modeled by different mathematical functions depending on the biological questions of interest. For the allometric scaling of traits, an exponential function is chosen (West et al. 1997). Growth equations—exponential, saturating, or sigmoidal—are often used to model ontogenetic development (Alberch et al. 1979).
Various polynomials prove to be powerful means of characterizing thermal reaction norms in organisms such as insects (Angilletta et al. 2003). We use a general function to express each of these three phenomena, that is,

\[ u = \begin{cases} 
  f(x) & \text{for allometric laws} \\
  g(t) & \text{for growth curves} \\
  h(z) & \text{for reaction norms}
\end{cases} \]

where \( u \) is a particular biological trait, \( x \) is the body size, \( t \) is the age, and \( z \) is an environmental variable such as temperature, nutrition, or light intensity. The forms of the mathematical functions, \( f(x) \), \( g(t) \), and \( h(z) \), can be linear or nonlinear.

By fitting the vector of genotypic means \( (u_j) \) in the equation above using the function values for different traits, for a single trait at different times, or for a single trait under different environmental conditions, predicted by \( f(x) \), \( g(t) \), and \( h(z) \), respectively, we have formulated a novel mapping model, called functional mapping, to map QTL of biological significance (Ma et al. 2002, 2003, Wu et al. 2002a, 2002b). Such a mapping model has been employed to map the QTL for allometric scaling and growth trajectories. In an example drawn from the published literature (Bradshaw and Stettler 1995, Wu and Stettler 1996), this model detected a QTL that affects allometric 1/4-power scaling of stem height and stem dry weight in a \( F_2 \) hybrid tree population derived from two different poplar species, \( P. trichocarpa \) and \( P. deltoides \) (Wu et al. 2002a). This QTL is located in a 5-cM (5-centi-Morgan)—wide interval bracketed by two molecular markers. Three genotypes of this QTL display significant differences in both stem height and biomass but experience the same scaling change in stem height as the 1/4 exponent of stem biomass. West and colleagues (1997) grounded their 1/4-scaling theory in the evolutionary optimization assumption, under which transport distance and transport time are minimized within the metabolic network. In nature, a strong scaling relationship exists between a tree's size and its physiological and life-history traits (Thomas and Bazzaz 1999, Reich 2000). Thus, the QTL identified in poplars may play an important role in the evolution of forest trees toward an optimal stem form for maintenance of mass at a minimum cost.

Similarly, a new model incorporating logistic growth laws was used to map QTL affecting ontogenetic trajectories in a second poplar hybrid population derived from \( P. deltoides \) and \( P. x euramericana \) (Ma et al. 2002). These two species were crossed to generate a pseudotest backcross progeny population (Yin et al. 2002), in which one of the parents was heterozygous but the other was null for a marker. This aspect of the pseudotest backcross design allows for the construction of two parent-specific linkage maps. A number of QTL that affect stem height and diameter growth processes were observed in different linkage groups in this mapping population, and the different patterns of the QTL expression as a function of age were identified. For a QTL found on a linkage group D7, two genotypes (QQ and Qq) display remarkably different stem height growth curves (figure 5). As indicated, the time \( t_{i1} \) and \( t_{i2} \) at which trees reach a maximum rate of height growth (the inflection point) is statistically different between the two QTL genotypes. Given that the inflection point reflects a tree's capacity to effectively acquire spatial resources, this QTL may be used to modify the tree's growth trajectory to better adapt to its competitive environment.

From growth curves associated with a QTL (e.g., figure 5), we can also find how genes control developmental timing. Many developmental events, such as time to first flower, age of maximum reproduction, or longevity, can be mapped on these growth curves. If there is a significant difference for the time to first flower between the QTL genotypes, as seen in figure 5, this QTL can be regraded as pleiotropic, as it not only affects growth curves but also triggers an effect on reproduction. Such a pleiotropic QTL, therefore, provides a unified view of growth and development, two physiological processes that have usually been separated in developmental studies.

Statistically, incorporation of these underlying laws into genetic models can potentially increase the power to detect a QTL and the precision of QTL localization in the genome. Simulation studies indicate that the precision of estimates of the QTL position and effect can be much improved when scaling relationships or ontogenetic trajectories of traits are considered (Ma et al. 2002, Wu et al. 2002a). We believe that this insight has opened up a new analytical approach to multitrait mapping studies. This approach should allow many biological and breeding questions, such as the nature of the genetic mechanisms of trait evolution and development, to be addressed directly.
Conclusion
The last decade has seen a renaissance in the study of evolutionary developmental biology, with particular emphasis on comparative analyses of the allometric scaling, ontogenetic histories, and phenotypic plasticity of organisms. In the past, evolutionary relationships between organisms focused only on individual adult phenotypes, and thus the adults of one species were seen as evolving from the adults of another species. However, the renewed interest in evolutionary developmental biology reveals that to fully understand how phenotypes evolve, researchers must look at the complete ontogenetic history of an organism and at its evolutionary relationships. We have developed a statistical method for incorporating the role of genetic determinants in the evolution of phenotypes. This is a step toward creating a unified view of developmental biology and evolutionary biology in the study of morphological evolution.

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References cited
Lark KG, Chase K, Adler F, Manur LM, Orf JH. 1995. Interactions between quantitative trait loci in soybean in which trait variation at one locus is conditional upon a specific allele at another. Proceedings of the National Academy of Sciences 92: 4656–4660.