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MODELING PHENOTYPIC PLASTICITY IN GROWTH TRAJECTORIES: A STATISTICAL FRAMEWORK

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Phenotypic plasticity, that is multiple phenotypes produced by a single genotype in response to environmental change, has been thought to play an important role in evolution and speciation. Historically, knowledge about phenotypic plasticity has resulted from the analysis of static traits measured at a single time point. New insight into the adaptive nature of plasticity can be gained by an understanding of how organisms alter their developmental processes in a range of environments. Recent advances in statistical modeling of functional data and developmental genetics allow us to construct a dynamic framework of plastic response in developmental form and pattern. Under this framework, development, genetics, and evolution can be synthesized through statistical bridges to better address how evolution results from phenotypic variation in the process of development via genetic alterations.

KEY WORDS: **Developmental trajectory, dynamic modeling, functional mapping, logistic growth curve, phenotypic plasticity.**

The capacity of an organism to alter its phenotypes in response to changing environment is known as phenotypic plasticity (Schlichting 1986; Sultan 2000). Because of its central role in evolution and speciation, an explosion of interest in studying the causes and consequences of this phenomenon has been enthused for over a century (Baldwin 1896; Waddington 1942; Scheiner 1993; Wu 1998; Agraval 2001; Pigliucci 2005; West-Eberhard 2005; Fusco and Minelli 2010; Nicotra et al. 2010; Pfennig et al. 2010; Sultan 2010; Beldade et al. 2011; Sommer and Ogawa 2011). Recently, mounting recognition has been gained for the fact that phenotypic plasticity may make an important contribution to the occurrence of complex human diseases (Bateson et al. 2004; Feinberg 2007; Burdge and Lillycrop 2010; Hochberg et al. 2011) and the response of biodiversity to climate change (Nicotra et al. 2010), stimulating the mechanistic study of plastic changes at the molecular and pathway level (Gilbert and Epel 2009).

The phenotypic plasticity of an organism arises from its genotype through programmed change in gene expression (Lewontin 2000; West-Eberhard 2005; Beldade et al. 2011). It has become increasingly clear that phenotypic plasticity is not only regulated by environmental factors, but also largely determined by endocrine hormones and epigenetic methylation that mediate various biological functions during development (Richards 2008; Sommer and Ogawa 2011). An emerging conceptual framework for studying phenotypic plasticity is to integrate it with development by viewing the phenotype as the outcome of complex synergistic developmental systems (Sultan 2000; Dmitriew et al. 2010; Love 2010; Beldade et al. 2011; Parsons et al. 2011). Fusco and Minelli (2010) assessed the value of taking the phenotypic plasticity of developmental processes into the picture to account for the evolution of life cycle.

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Unlike static phenotypes at single time points, the phenotypic plasticity of development entails the dynamic modeling of a series of phenotypes measured in a time course (Wu and Lin 2006; Li and Wu 2010; Li et al. 2010b; He et al. 2010.). The advantages of such modeling are twofold. First, it allows biological principles of trait formation and progression to be incorporated through robust mathematical equations into the analysis of environmental sensitivity (West et al. 2001). A number of quantitative hypotheses can be made for the interplay between environment and development in a hope to address fundamental questions in biology; for example, how the environment affects developmental rate and timing and the length of a particular developmental event in the lifetime of an organism (Parsons et al. 2011) and how the environment guides the development of traits to achieve maximum fitness (Agraval 2001; Beldade et al. 2011). Second, statistical modeling of developmental traits is based on a few parsimonious parameters that can capture the structure of trait development and correlation, thus facilitating the computation of a complex model and its power for the detection of environment-induced differences (Ma et al. 2002; Griswold et al. 2008).

Analytical approaches for modeling dynamic traits are not new in the literature (Griswold et al. 2008). Kirkpatrick and Heckman (1989) provided a general framework for studying variation and evolution of functional traits. Kirkpatrick et al. (1990) and Kirkpatrick and Lofsvold (1992) used functional data analysis approaches to analyze growth trajectories, and Gomulkiewicz and Kirkpatrick (1992) did the same for reaction norms across a gradient of environments. Izem and Kingsolver (2005) and Griswold et al. (2008) explored testing a priori hypotheses for growth trajectories and reaction norms, considering basis function and parametric approaches. Stinchcombe et al. (2010) used logistic growth models to analyze variation and plasticity of growth rate in *Impatiens capensis*. In a recent review, Stinchcombe et al. (2012) identified more than 30 studies that have used functional data analysis approaches to test ecological and evolutionary hypotheses about plasticity, developmental trajectories, and other "function-valued" traits.

Despite these extensive developments, a synthetic review of these analytical models, their theoretical ground, applications, and interpretations, has not been made. In this article, we describe a general framework for studying the phenotypic plasticity of developmental trajectories by integrating mathematical aspects of phenotypic differentiation. We show how this framework can be used to test and quantify environmental impact on the pattern of development. We further investigate by which environmental change alters the timing of development, its rate and even its direction. We provide an outlook on how the dynamic framework enables quantitative geneticists and evolutionary biologists to jointly construct and materialize a synthetic theory of evo-devo.

Developmental Plasticity: Types and Components

Phenotypic plasticity can be classified in different ways; for example, based on the nature of the trait (e.g., morphological, physiological, or behavioral; Sultan 2000) or environmental cue (e.g., temperature, diet, or photoperiod) considered (Schlichting 1986). Also, as the capacity to modify developmental trajectories in response to specific environmental cues, plasticity phenomena can be distinguished in terms of specific phases of development which are sensitive to the environment (Fusco and Minelli 2010). For example, a turtle determines the sex based on the degree of temperature change it faces in its embryonic stage (Crews 2003), whereas the migratory grasshopper displays population density-dependent wing polyphenisms during postembryonic nymphal stages (Applebaum and Heifertz 1999). Different from this qualitative description, we classify environment-induced changes for development in a quantitative way:

(a) ***Early-late plasticity*:** Environmental change affects the time at which the development starts, but has no effect on the rate of growth, with the pattern and form of developmental processes unchanged. As shown in Figure 1A, the trait begins developing earlier in the new environment than it did in the original one.

(b) ***Slow-fast plasticity*:** Environmental change triggers an effect on the rate of growth rather than on the starting point of development. In Figure 1B, the trait is shown to develop at a higher rate in the new environment than in the original one.

(c) ***Short-long plasticity*:** The environment changes the time at which the development ceases, although it does not alter developmental trajectory. In Figure 1C, the development of the trait considered in the new environment continues beyond the point at which it stopped in the original environment.

(d) ***Sequential plasticity*:** Environmental change leads to the change of sequential differentiation in early and late stages. As shown in Figure 1D, during the early stage the trait develops at a lower rate in the new environment than in the original one, but this is inverted during the late stage.

Each of these developmental plasticity types may play a different role in regulating phenotypic variation and evolution, but no analytical model is available to discern these distinct roles and, more importantly, to synthesize and organize different roles into a unify framework.

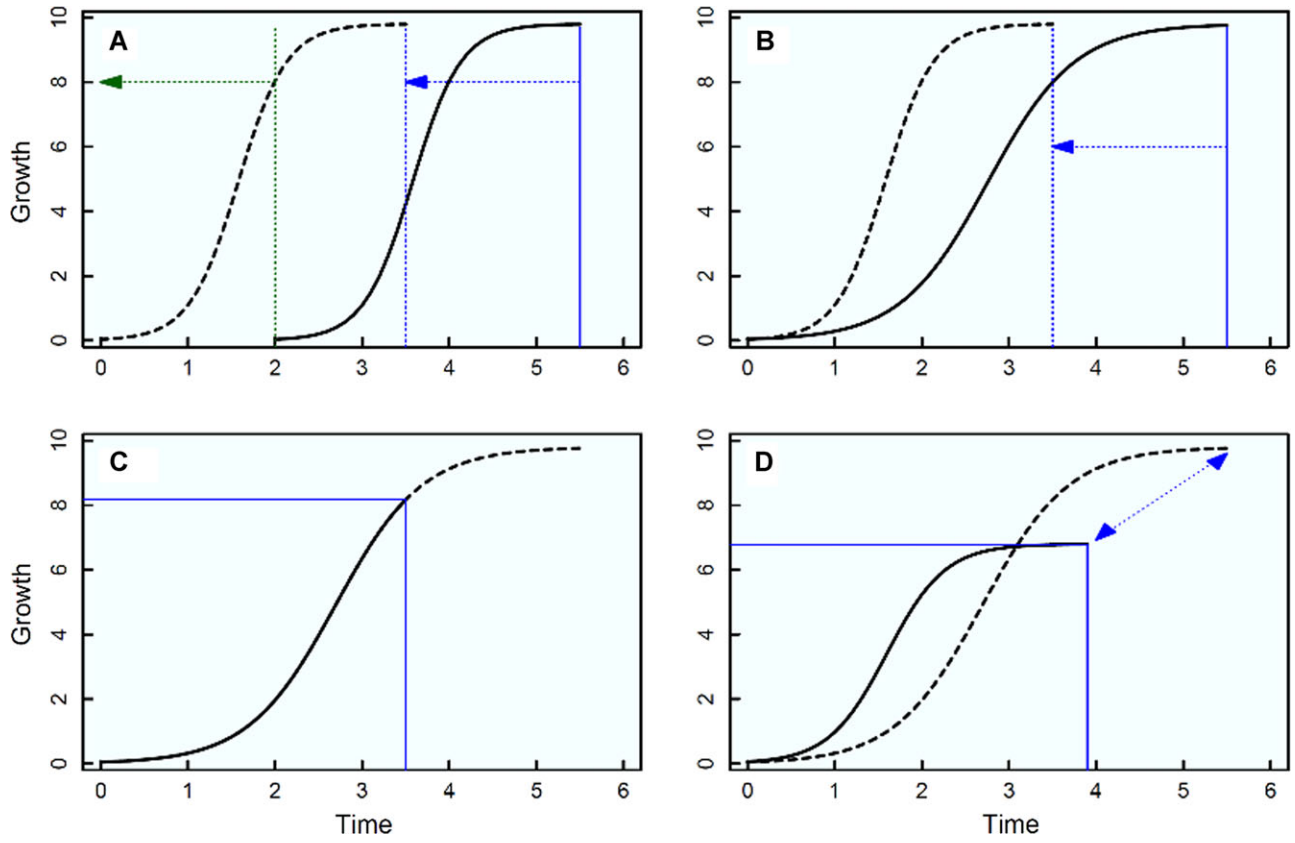

Figure 1. Four representative types of developmental plasticity which arises from the transplantation of the same genotype from its original environment (solid) to a new environment (dash). (A) Early-late plasticity, in which development starts and stops earlier (shown by arrows) in response to environmental change. (B) Slow-fast plasticity, in which the rate of growth is accelerated in the new environment, thus using a shorter time to achieve the maximum growth (shown by an arrow). (C) Short-long plasticity, in which growth is prolonged in the new environment (shown by a dash curve). (D) Sequential plasticity, in which the environment leads to decreasing growth in the early stage of development but increasing growth in the late development.

How to Quantify the Phenotypic Plasticity of Development

FUNCTIONAL MODEL

Plastic response of a trait to the changing environment occurs not by the direct transformation of its adult phenotype from the original environment to the new environment but rather when a series of environment-specific features are produced in the process of development. Therefore, phenotypic plasticity cannot be understood without understanding the plasticity of developmental trajectory. To study developmental plasticity, different clonal or inbred replicates of the same genotype are grown in multiple distinct environments. The growth of each replicate in each environment is measured repeatedly at multiple time points during the lifetime (Parsons et al. 2011).

Univariate repeated-measures ANOVA or multivariate analysis can be used to analyze and compare across-environment differences of longitudinal mean phenotypes by treating functional data as a vector of phenotypic responses (Johnson and Wichern 1988). However, these approaches do not explicitly consider the ordering and spacing of time points of an individual's response and also fail to model the intrinsic biological properties of trait development. Indeed, the growth of many traits, such as plant height or cancer size, follows a logistic curve which is derived from fundamental principles of biophysics and biochemistry (West et al. 2001). One mathematical form of the logistic curve is expressed as

$$g(t) = a(1 + be^{-rt})^{\frac{1}{1-k}}, \quad (1)$$

where $g(t)$ is the trait value at time t, a is the asymptotic value of the trait, b is a parameter to position the curve on the time axis, r is the growth rate constant of the trait, and k is the shape parameter of the curve. Thus, environment-induced changes in overall and specific features of development can be captured by estimating the set of parameters (a,b,r,k) that specifies the growth equation (1).

By fitting the growth equation (1) to observed longitudinal data within a likelihood setting (Appendix 1), we can obtain the maximum likelihood estimates (MLEs) of the parameters (a,b,r,k)

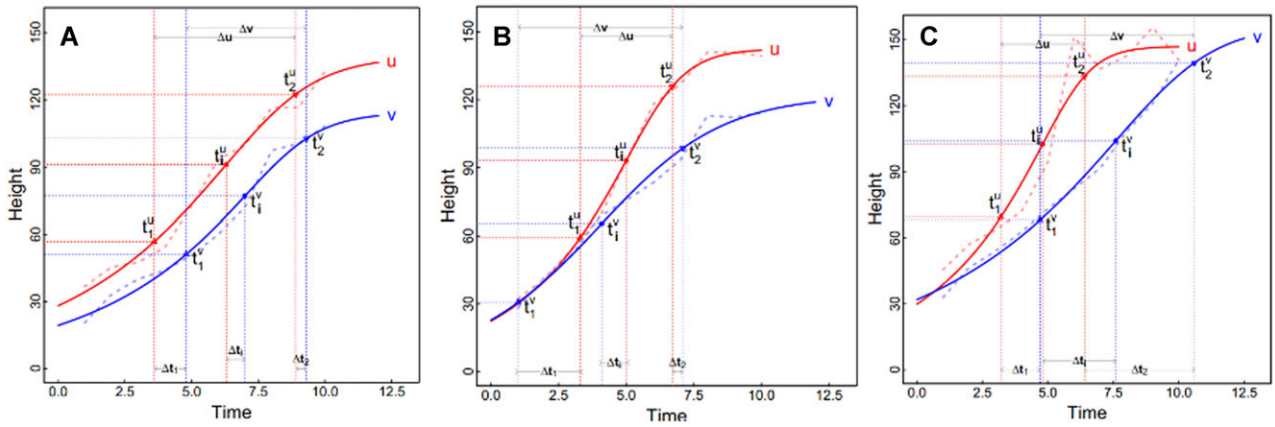

Figure 2. **Phenotypic plasticity of developmental trajectories for three rice lines from a mapping population, recombinant inbred lines (RIL) 8 (A), RIL 102 (B), and RIL 19 (C), between two contrasting environments, tropical Hainan (*u*) and subtropical Hangzhou (*v*). Observed growth data over time (dashed line) were fit by growth equation (1) (solid line). The timing of three developmental landmarks, the inflection point of growth curve, the first inflection point of growth rate curve, and the second inflection point of growth rate curve, is denoted as (t_i^u,t_i^v), (t_1^u,t_1^v), and (t_2^u,t_2^v) for the two environments, respectively. The phenotypic plasticity of these landmarks is expressed as $\Delta t_i = t_i^u - t_i^v$, $\Delta t_1 = t_1^u - t_1^v$, and $\Delta t_2 = t_2^u - t_2^v$, respectively. The phenotypic plasticity of the time duration for the linear growth phase or grand period of growth can be tested by comparing $\Delta u = t_2^u - t_1^u$ with $\Delta v = t_2^v - t_1^v$.**

for different environments. Figure 2 gives an example of curve fitting for plant height growth of three recombinant inbred lines (RILs) selected from a mapping population of rice planted in two contrasting environments, tropical Hainan (u) and subtropical Hangzhou (v) (Yan et al. 1998; Zhao et al. 2004a,b). The MLEs of two sets of parameters $\mathbf{\Theta}_u = (a_u,b_u,r_u,k_u)$ and $\mathbf{\Theta}_v = (a_v,b_v,r_v,k_v)$ were obtained for plant height growth in the two environments, respectively. By testing how $\mathbf{\Theta}_u$ is different from $\mathbf{\Theta}_v$, we can determine whether and how the environment impacts developmental trajectories in rice plant height. RIL 8 displays better plant height growth during all stages of development in Hainan than Hangzhou (Fig. 2A), but RIL 102 performs better only in the middle to late stage of growth (Fig. 2B). Despite reaching a similar adult height in both environments, RIL 19 uses different pathways (Fig. 2C). Environment-induced variation in developmental trajectory for these rice lines can be explained by one or mixed types of developmental plasticity as shown in Figure 1.

MECHANISTIC TESTS OF DEVELOPMENTAL TIMING AND RATE

The growth equation (1) has several features that can fully describe the pattern and form of developmental process. There are three physiologically important points, with coordinates denoted as P_1, P_i, and P_2, respectively, on the growth curve (Fig. 3A). The point P_i, known as the inflection point, is one at which growth rate reaches its maximum (Fig. 3B). Because of this point, the curve is divided into two phases, the exponential growth (from time $t = 0$ to P_i) and the asymptotic growth (from P_i to the infinite time). Thus, the determination of the timing of the inflection point can help to better understand the shape and process of trait growth. The points P_1 and P_2 present the timing of maximum acceleration and maximum deceleration of growth (Fig. 3C), which are the first and second inflection points of growth rate curve, respectively. These two points partition the growth curve into three phases, the exponential growth (from time $t = 0$ to P_1), the linear growth (from P_1 to P_2), and the ageing (from P_2 to the infinite). The coordinates of these three points, expressed as (t_1,g_1), (t_i,g_i), and (t_2,g_2), respectively, can be obtained by calculating the first, second, and third derivatives of the growth equation (1) with respect to time.

In Appendix 1, we provide the mathematical expressions for the coordinates of three points P_1, P_i, and P_2. Based on these expressions, we can estimate MLEs of the coordinates. Furthermore, the phenotypic plasticity of these coordinates that play a key role in shaping developmental processes can be tested by formulating a log-likelihood ratio approach. Through these tests, we can address the following questions:

Question 1: Whether does the environment affect the timing and amount of maximum acceleration for development? This can be tested by formulating a null hypothesis H_0: $(t_1^u,g_1^u) = (t_1^v,g_1^v)$. As shown in Table 1, the environment has a significant effect on these two parameters for RIL 102.

Question 2: Whether does the environment affect the timing and amount of maximum deceleration for development? This can be tested by formulating a null hypothesis H_0: $(t_2^u,g_2^u) = (t_2^v,g_2^v)$. These two parameters are significantly different between the two environments for RIL 19 (Table 1).

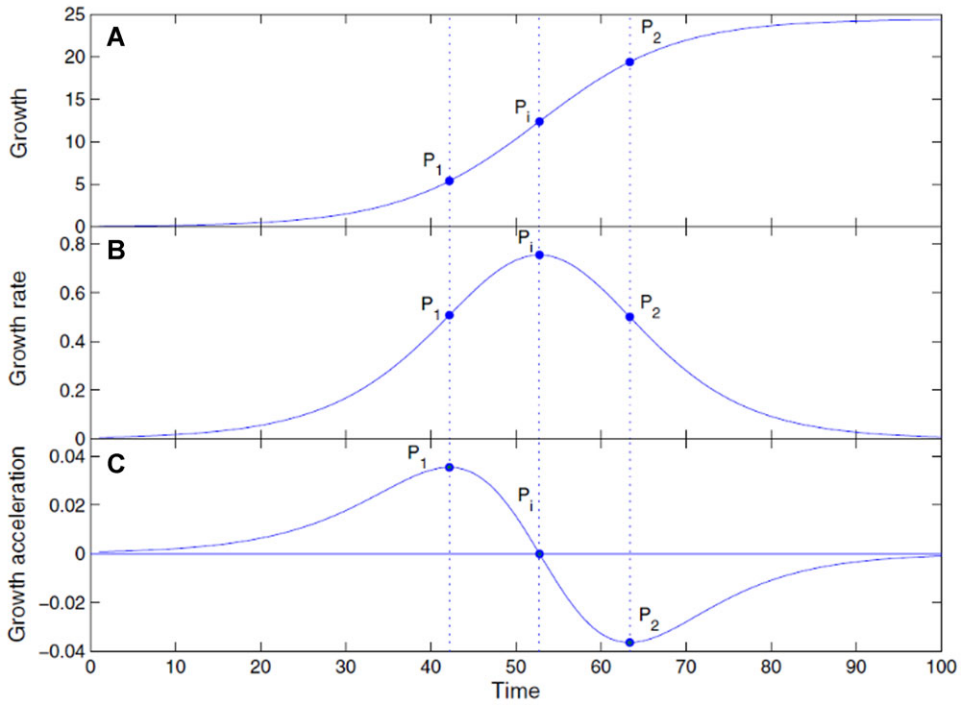

Figure 3. Growth curve (A), growth rate curve (B), obtained from the derivative of growth curve, and growth acceleration curve (C), obtained from the derivative of growth rate curves. Three critical points P_1, P_i, and P_2 are shown on the curves.

Question 3: Whether does the environment affect the timing of the inflection point and its amount of growth? This can be tested by formulating a null hypothesis H_0: $(t_i^u, g_i^u) = (t_i^v, g_i^v)$. These two parameters are significantly different between the two environments for RIL 102 (Table 1).

Question 4: Whether does the environment affect the time duration of the linear growth phase? This can be tested by formulating a null hypothesis H_0: $\Delta_u = t_2^u - t_1^u = t_2^v - t_1^v = \Delta_v$. RIL 102 and 19 each experience different lengths of linear growth between the two environments (Table 1). The impact of environment on the growth of the linear growth phase can also be tested by using H_0: $g_2^u - g_1^u = g_2^v - g_1^v$.

Question 5: Whether does the environment affect the rate of growth? This can be tested by formulating a null hypothesis H_0: $r_u = r_v$. All three RIL have different growth rates (Table 1).

The change in developmental rate and timing is thought of as a special means of producing novelty by simple changes in

Table 1. Analysis of developmental plasticity for plant height for three rice recombinant inbred lines (RIL) grown in two different environments, Hainan (*u*) and Hangzhou (*v*).

	RIL 8		RIL 102		RIL 19	
	u	*v*	*u*	*v*	*u*	*v*
Growth parameter						
a	139	114	142	122	147	157
b	254	2142	972	9	2514	535
r	0.6868	0.8816	1.1061	0.4600	1.3003	0.6423
k	4.4617	5.31	4.72	2.36	5.92	4.95
Developmental timing (coordinates)						
t_1	$(3.6^a, 56^a)$	$(4.8^b, 51^a)$	$(3.3^a, 59^a)$	$(1.0^b, 31^b)$	$(3.2^a, 69^a)$	$(4.7^b, 68^a)$
t_i	$(6.8^a, 91^a)$	$(7.0^a, 77^b)$	$(5.0^a, 93^a)$	$(4.1^b, 65^b)$	$(4.8^a, 102^a)$	$(7.6^b, 104^a)$
t_2	$(8.9^a, 122^a)$	$(9.3^a, 103^b)$	$(6.7^a, 126^a)$	$(7.1^a, 98^b)$	$(6.4^a, 133^a)$	$(10.6^b, 140^b)$
Δ	5.3^a	4.7^a	3.3^a	6.1^b	3.2^a	5.9^b

Note: The significance test, indicated by the letter at the superscript of a parameter, is made for each parameter in the vector (·, ·) between two environments *u* and *v*.

development because an entire organism can be affected by a single perturbation of any one of these two parameters (Gould 1977). Thus, the quantitative test of environment-induced difference in the timing, rate, and length of development can enhance our understanding of the developmental mechanisms underlying the evolutionary adaptation of organisms to different environments.

NONPARAMETRIC MODELING

If the mathematical function underlying the time-dependent change of a trait is unknown, nonparametric methods can be used to analyze the functional data. In nonparametric methods (Ramsay and Silverman 2005), we use basis function expansions to approximate any function of biological interest at any degree of precision. These basis function expansions are constructed from various families of basis functions including splines, Fourier series, and the Legendre polynomials (Wu et al. 2007b). For a practical data set, model selection criteria are needed to determine which models, parametric or nonparametric, can better explain the longitudinal data and show the largest power for detecting phenotypic differences among environments.

If a nonparametric model is finally used, we can still not only test the environment-induced differences of overall developmental plasticity curves, but also identify whether the environmental influence play an important role in producing the difference in the rate and timing of development. As a case for the growth equation (1), this identification procedure can be based on the test of P_1, P_i, and P_2 obtained from the first, second, and third derivatives of the basis function expansion with respect to time. A detailed formulation of nonparametric approaches deserves further investigation.

Developmental Trajectories of Phenotypic Plasticity

We have discussed how an organism changes its developmental trajectories to adapt changing environment. In reality, the change of developmental pathways triggered by environmental cues may be induced during a certain sensitive periods of the organism's development (Sultan 2000; Bateson et al. 2004; Hovermana and Relyea 2007; Burggren and Reyna 2011). Outside these sensitive periods an environmental influence may have little or no effect on the characteristics of the organism (Bateson and Martin 1999). Plasticity being restricted to a particular period of life may be explained by the difficulties of reversing developmental processes or the costs in terms of survival or reproductive success of changing the adult characteristics of the organism (Lindstrom 1999; Bateson 2001). A direct approach for identifying such development windows of phenotypic plasticity is to propose a concept, called the developmental trajectory of phenotypic plasticity, to better study how phenotypic plasticity is under developmental control.

Consider a genotype planted in two different environments u and v with replicates. A phenotypic trait is measured in both environments at multiple time points (say T) in ontogeny. Let $y_u(1), \ldots, y_u(T)$ and $y_v(1), \ldots, y_v(T)$ denote the vector of phenotypic values in environment u and v, respectively. The developmental trajectory of phenotypic plasticity is expressed, in vector form, as

$$z_1 = (y_u(1) - y_v(1), \ldots, y_u(T) - y_v(T)) \text{ for the difference model,} \quad (2)$$

or

$$z_2 = (y_u(1)/y_v(1), \ldots, y_u(T)/y_v(T)) \text{ for the difference model,} \quad (3)$$

These two models capture different features of time-dependent phenotypic plasticity. The difference model explains the absolute change of phenotypic plasticity over time, whereas the ratio model considers the relative change of the trait value from one environment to next in a time course. It is possible that there is no explicit parametric function underlying the difference (2) or ratio data (3). In this case, the longitudinal difference and ratio data should be modeled by a nonparametric approach based on splines, Fourier series, or Legendre polynomials.

The significance of phenotypic plasticity in the entire time course can be tested by using the null hypothesis H_0: $z_1(t) = 0$ for the difference model or $z_2(t) = 1$ for the ratio model. This is a global test. If the null hypothesis of this global test is rejected, we then scan the test of phenotypic plasticity at a particular time t from time 1 to T. Through this local test, one can draw a dynamic picture of when the environment starts and stops exerting its effect on plastic change and how long the environment affects phenotypic plasticity. This test procedure will have many implications in practice. For example, using this picture, wildlife ecologists can establish developmental windows during which a prey alters their phenotypes to form a defense to predation risk (Hovermana and Relyea 2007).

Genetic and Epigenetic Mechanisms Underlying Developmental Plasticity

GENETIC MAPPING

An increasing body of evidence has shown that phenotypic plasticity is controlled by genetic and epigenetic factors (Wu 1998; Feinberg 2007; Lacaze et al. 2009; Beldade et al. 2011; Wang et al. 2013). Functional mapping has proved to be a powerful tool for detecting quantitative trait loci (QTLs) that control developmental trajectories by integrating mathematical functions of

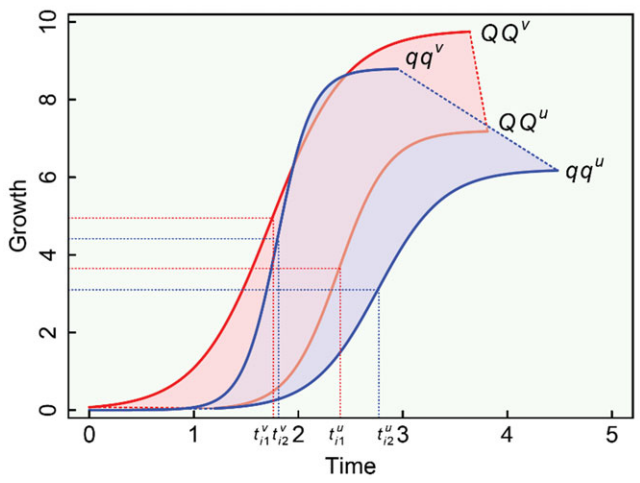

Figure 4. Genetic control of developmental plasticity to two contrasting environments *u* and *v* by a quantitative trait locus (QTL). Genotype *QQ* is consistently better than genotype *qq* in environment *u*, but in environment *v*, the former surpasses the latter in early and late stages of development, both with a similar growth in the middle stage. Although this QTL affects developmental trajectories in both environments, its pattern of action is highly environment specific. The huge difference in the shape and size of the areas covered by genotypes *QQ* (red) and *qq* (blue) from environment *v* to *u* indicates that this QTL has remarkable genotype–environment interactions for developmental trajectories. The way of how this QTL affects the time of the inflection point is also indicated for each environment.

development into a mixture model framework (Ma et al. 2002; Wu et al. 2007b; Wu and Lin 2006; Li and Wu 2010). Functional mapping allows developmental changes in genetic effects and variances resulting from specific QTLs to be identified. Zhao et al. (2004a,b) extended functional mapping to dissolve genotype–environment interactions into individual genetic loci for developmental processes. This extended model allows the characterization of how individual QTLs interact with the environment to determine overall developmental trajectory curves and the rate and timing of development.

Appendix 2 illustrates the procedure of functional mapping to map QTLs that control environment-induced differences in developmental plasticity. Assume that we detected a QTL for plant height growth trajectories in a doubled haploid population of rice grown in tropic subtropical Hangzhou (u) and Hainan (v). Functional mapping obtains the MLEs of the growth curve parameters for two QTL genotypes QQ (coded as 1) and qq (coded as 2), $\mathbf{\Theta}_{1u} = (a_{1u},b_{1u},r_{1u},k_{1u})$ and $\mathbf{\Theta}_{2u} = (a_{2u},b_{2u},r_{2u},k_{2u})$, in Hangzhou and, $\mathbf{\Theta}_{1v} = (a_{1v},b_{1v},r_{1v},k_{1v})$ and $\mathbf{\Theta}_{2v} = (a_{2v},b_{2v},r_{2v},k_{2v})$, in Hainan. Using these MLEs, four genotype- and environment-dependent curves are drawn in Figure 4, from which a number of hypotheses can be tested.

1. This QTL affects plant growth trajectories, which can be tested on the basis of the null hypothesis H_0: $\mathbf{\Theta}_{1u} = \mathbf{\Theta}_{2u}$ and $\mathbf{\Theta}_{1v} = \mathbf{\Theta}_{2v}$. The rejection of the H_0 supports the hypothesis of QTL existence. Two genotypes QQ and qq, diagrammed in Fig. 4, are different in each environment, suggesting that this QTL is significantly associated with plant height growth trajectories.
2. This QTL affects the phenotypic plasticity of overall plant height growth trajectories. The null hypothesis for this statement is H_0: $\mathbf{\Theta}_{1u} - \mathbf{\Theta}_{2u} = \mathbf{\Theta}_{1v} - \mathbf{\Theta}_{2v}$. In Figure 4, the differences of the two QTL genotypes are not the same between the two environments, suggesting significant QTL–environment interactions for height growth.
3. This QTL affects the phenotypic plasticity of the inflection point, that is, the time of maximal growth rate. This can be tested using the null hypothesis, H_0: $t^u_{i1} - t^u_{i2} = t^v_{i1} - t^v_{i2}$. Figure 4 shows that the inflection point is not very different between the two genotypes in Hainan, but it differs strikingly in Hangzhou, suggesting that this QTL determines the plastic response of the inflection point.
4. Other hypotheses include whether this QTL affects the phenotypic plasticity of the timing of maximum acceleration and maximum deceleration, growth rate, and the length of linear growth in plant height.

By calculating the genetic variance due to the QTL using the estimated curve parameters of two genotypes QQ and qq, we can characterize ontogenetic changes of how the QTL controls growth. Ontogenetic patterns in the genetic variance of a trait are influenced by resource conditions and selection pressure experienced during development (Cheverud et al. 1983; Riska et al. 1984; Wilson et al. 2005; Dmitriew et al. 2010). By studying the temporal pattern of how genetic components change with an organism's age, we can better understand the evolution of development, enriching, and deepening the content of developmental evolutionary biology (evo-devo; Nijhout and Emlen 1998; Raff 2000; Muller 2007; Carroll 2008; Rice 2008). However, this study should incorporate environmental influence because the environment in which growth occurs might mediate the change of genetic variance in ontogeny. For example, the genetic variance that is hidden in benign conditions (cryptic genetic variance) may be activated during development, when the growth is exposed to stress or novel environments (Dmitriew et al. 2010). This hypothesis can be tested by comparing the ontogenetic change of genetic variance exerted by the QTL between different environments, for example, Hainan and Hangzhou in the example of Figure 4.

EPIGENETIC MAPPING

Development is under epigenetic control through heritable changes in gene function that occur independently of alterations to primary DNA sequence (Bateson et al. 2004; Feinberg and

Irizarry 2010). It is crucial that global QTL mapping analyses are integrated with epigenetic mapping to decipher a detailed picture of the genetic and epigenetic architecture of developmental trajectories. By assuming that allelic DNA sequence variation is partly responsible for the trait variation, QTL mapping aims to deduce the locations of the contributing QTLs. Epigenetic mapping attempts to associate inheritance of the trait with segregation of informative epigenetic polymorphisms, or *epialleles* (Johannes et al. 2008; Feinberg and Irizarry 2010). Thus, QTL and epigenetic mapping are complementary, but the latter has several advantages. QTL mapping is limited in resolution because of meiotic recombination and population size, placing quantitative traits on genomic regions that are each typically several megabase-pairs long, and requires DNA sequence variation. In contrast, epigenetic mapping can make use of powerful emerging mapping techniques that allow the positioning of epialleles defined by chromatin variation to individual genes or chromosomal regions, even in the absence of DNA sequence variation. There is a successful example for the epigenetic mapping of enhancers of the $Kr^{\text{If-1}}$ ectopic eye bristle phenotype in an isogenic strain of *Drosophila melanogaster* (Garfinkel et al. 2004).

Statistical models for functional mapping (Appendix 2) can be incorporated with epigenetic data, allow genomic regions of DNA methylations for developmental trajectories and developmental plasticity to be identified and quantified. In the new model, the novel hypothesis can be formulated about whether an epiallele affects the developmental process of phenotypic plasticity and how it interacts with QTLs to determine this phenomenon. This can be made possible by integrating the developmental model of phenotypic plasticity and a quantitative epigenetic model proposed by Wang et al. (2013).

Synthesizing Developmental Plasticity and Omics

Biologists are increasingly aware about the importance of linking development with phenotypic plasticity because environmentally induced phenotypes require time to form and build (Nicoglou 2011). Compared to the study of conventional static traits, more sophisticated mathematical and statistical tools are entailed for modeling the phenotypic plasticity of developmental trajectory. The number and complexity of such tools can be made available to meet a growing number of genetic, ecological, and evolutionary studies in developmental plasticity through integrating mathematical models within the biological context of this phenomenon. In this article, we have made a first exploration of how to study and quantify developmental trajectory, its genetic causes and evolutionary consequences, using a dynamic modeling framework. The framework is demonstrated by applying it to analyze the phenotypic plasticity of stem height growth trajectories in rice plants grown in two contrasting environments. The plastic response of developmental timing, developmental period and developmental pattern has been precisely quantified for individual genotypes.

The framework can be integrated with genetic mapping approaches. By associating marker information genotyped for a mapping population with developmental plasticity, the extended framework allows the identification of specific genes and their genetic interactions that control variation in developmental plasticity, affording an unprecedented understanding of how the control of developmental processes is triggered by the interplay between genetic, epigenetic, and environmental factors. With the increasing availability of "omics" data, the framework may play a more important role in synthesizing developmental plasticity with network biology (de Bruijn et al. 2012). This synthesis enables the unveiling of the regulatory control mechanisms for the formation and evolution of complex phenotypes in response to environmental changes and facilitates the construction of a new evolutionary developmental biology (evo-devo) theory concerned with the discovery and understanding of the changes in developmental mechanisms and their role in the evolutionary origin of aspects of the phenotype.

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Appendix 1

STATISTICAL TESTS OF THE PHENOTYPIC PLASTICITY OF DEVELOPMENT

Consider a range of environments in which a species considered can grow. Now, there are L such environments in each of which we grow the same genotype with M clonal or inbred replicates. Each replicate is measured for a phenotypic trait at a series of T time points during its ontogenetic development. Let $\mathbf{y}_{lm_l} = (y_{lm_l}(1), \ldots, y_{lm_l}(T))$ denote a vector of time-dependent phenotypic values of the trait for replicate m_l ($l = 1, \ldots, L$; $m = 1, \ldots, M$) in environment l. Assume that the trait changes its value over time in a particular function, such as growth equation (1), thus the time trajectory of trait value can be described by estimating and testing the parameters that define the function.

To estimate these parameters in individual environments, we construct a likelihood for time-dependent phenotypic data, expressed as

$$L(\mathrm{y}_1, \ldots, \mathrm{y}_L) = \prod_{l=1}^{L} \prod_{m=1}^{M_l} f_l(\mathbf{y}_{lm_l}), \tag{A1}$$

when L environments are assumed to be independent of each other, where $f_l(\mathbf{y}_{lm_l})$ is a multivariate normal distribution of time-dependent trait values for replicate m_l in environment l, that is,

$$f_l(\mathbf{y}_{lm_l}) = \frac{1}{(2\pi)^{T/2}\,|\Sigma_l|^{1/2}} \exp\left(-\frac{1}{2}(\mathbf{y}_{lm_l} - \mu_l)'\Sigma_l^{-1}(\mathbf{y}_{lm_l} - \mu_l)\right)$$

with mean vector $\boldsymbol{\mu}_l$ and covariance matrix $\boldsymbol{\Sigma}_l$.

We will use a mathematical function derived from fundamental principles of biology to specify the mean vector and autoregressive models to specify the covariance structure. Considering the growth equation (1), for example, we have

$$\begin{aligned}\boldsymbol{\mu}_l &= [\mu_l(1), \ldots, \mu_l(T)] \\ &= \left[a_l(1 + b_l e^{-r_l})^{\frac{1}{1-k_l}}, \ldots, a_l(1 + b_l e^{-r_l T})^{\frac{1}{1-k_l}}\right].\end{aligned} \tag{A2}$$

Thus, the growth trajectory of a genotype in environment l can be described by parameter set (a_l, b_l, r_l, k_l). The covariance structure $\boldsymbol{\Sigma}_l$ can also be modeled by a parsimonious and flexible approach such as an autoregressive, antedependence, autoregressive moving average, or nonparametric and semiparametric approaches (Li et al. 2010a). The EM algorithm can be implemented to obtain the MLEs of the parameters that specify the mean vector and covariance structure.

Using the MLEs of growth parameters (Table 1), one can formulate various hypotheses tests for the phenotypic plasticity of developmental pattern and form. Figure 2 describes an example of analyzing developmental plasticity for plant height growth in three different rice genotypes each grown in two sharply contrasting environments, tropical Hainan (u) and subtropical Hangzhou (v). For each environment, the coordinates of three developmental landmarks, P_i (the inflection point of growth curve), P_1 (the first inflection point of growth rate curve), and P_2 (the second inflection point of growth rate curve), were calculated as

$$\left(t_i^l, g_i^l\right) = \left(\frac{1}{r_l}\ln\left[\frac{b_l}{k_l - 1}\right], a_l k_l^{\frac{1}{1-k_l}}\right), \tag{A3}$$

$$\left(t_1^l, g_1^l\right) = \left(t_i^l + \frac{1}{r_l}\ln\left[\frac{(k_l - 1)\eta_{1l}^{k_l - 1}}{1 - \eta_{1l}^{k_l - 1}}\right], a_l\eta_{1l}\right), \tag{A4}$$

$$\left(t_2^l, g_2^l\right) = \left(t_i^l + \frac{1}{r_l}\ln\left[\frac{(k_l - 1)\eta_{2l}^{k_l - 1}}{1 - \eta_{2l}^{k_l - 1}}\right], a_l\eta_{2l}\right), \tag{A5}$$

where

$$\eta_{1,2l} = \left[\frac{k_l(k_l + 1) \mp (k_l - 1)\sqrt{k_l(k_l + 4)}}{2k_l(2k_l - 1)}\right]^{\frac{1}{k_l - 1}}.$$

Based on these expressions, we can address five fundamental questions given in section "Mechanistic Tests of Developmental Timing and Rate."

Appendix 2

A MODELING FRAMEWORK FOR FUNCTIONAL MAPPING OF DEVELOPMENTAL PLASTICITY

Consider a mapping population of n progeny each with multiple replicates grown in L environments. The mapping population is genotyped using a panel of molecular markers that cover the genome. All individuals are phenotyped for a phenotypic trait at a series of T time points. If specific QTLs exist to affect the dynamic change of the trait, the parameters that specify the change should be different among QTL genotypes. Genetic mapping uses a mixture model-based likelihood to estimate QTL genotype-specific parameters. Taking the means of replicates for each progeny in each environment, this likelihood is expressed as

$$L(\mathrm{y}_1, \ldots, \mathrm{y}_L) = \prod_{l=1}^{L} \prod_{i=1}^{n} \left[\omega_{1|i} f_{l1}(\mathbf{y}_{li}) + \ldots + \omega_{J|i} f_{lJ}(\mathbf{y}_{li})\right], \tag{A6}$$

where $\mathbf{y}_{li} = (y_{li}(1), \ldots, y_{li}(T))$ is the phenotypic mean vector of progeny i measured at T time points in environment l, $\omega_{j|i}$ is the conditional probability of QTL genotype j ($j = 1, \ldots, J$) given the marker genotype of individual i, $f_{lj}(\mathbf{y}_{li})$ is a multivariate normal distribution with expected mean vector for genotype j in environment l,

$$\mu_{ij} = (\mu_{lj}(1), \ldots, \mu_{lj}(T)) \tag{A7}$$

and covariance matrix $\mathbf{\Sigma}_l$.

The likelihood (A6) contains the conditional probabilities of QTL genotypes given marker genotypes, which can be expressed as a function of recombination fractions for an experimental cross population or linkage disequilibria for a natural population (Wu et al. 2007a). The EM algorithm can be implemented to estimate the recombination fractions or linkage disequilibria and, therefore, the positions of QTLs throughout the genome.

If a growth trait is considered, then the mean vector (A7) can be modeled by growth equation (1) in a similar way like equation (A2). Each QTL genotype j has a set of growth parameters (a_{lj}, b_{lj}, r_{lj}, k_{lj}) for each environment l, from which we can test how the QTL affects the phenotypic plasticity of developmental timing, pattern and process. For example, to test whether the QTL affects the phenotypic plasticity of the inflection point between Hainan (u) and Hangzhou (v), we can formulate the null hypothesis as follows:

$$\mathrm{H}_0: \frac{1}{r_{u1}} \ln\left[\frac{b_{u1}}{k_{u1}-1}\right] - \frac{1}{r_{u2}} \ln\left[\frac{b_{u2}}{k_{u2}-1}\right] = \frac{1}{r_{v1}} \ln\left[\frac{b_{v1}}{k_{v1}-1}\right] - \frac{1}{r_{v2}} \ln\left[\frac{b_{v2}}{k_{v2}-1}\right] \tag{A8}$$

$$\mathrm{H}_0: a_{u1} k_{u1}^{\frac{1}{1-k_{u1}}} - a_{u2} k_{u2}^{\frac{1}{1-k_{u2}}} = a_{v1} k_{v1}^{\frac{1}{1-k_{v1}}} - a_{v2} k_{v2}^{\frac{1}{1-k_{v2}}} \tag{A9}$$

where we assume that there are two genotypes 1 and 2 at the QTL considered. The rejection of the null hypothesis (A8) and (A9) indicates that the QTL has a significant effect on the timing and growth of the inflection point, respectively. The hypotheses about the genetic control of the plasticity of other developmental landmarks can be tested in a similar way.