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# DEVELOPMENTAL INTERACTIONS AND THE CONSTITUENTS OF QUANTITATIVE VARIATION

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*Abstract.*—Development is the process by which genotypes are transformed into phenotypes. Consequently, development determines the relationship between allelic and phenotypic variation in a population and, therefore, the patterns of quantitative genetic variation and covariation of traits. Understanding the developmental basis of quantitative traits may lead to insights into the origin and evolution of quantitative genetic variation, the evolutionary fate of populations, and, more generally, the relationship between development and evolution. Herein, we assume a hierarchical, modular structure of trait development and consider how epigenetic interactions among modules during ontogeny affect patterns of phenotypic and genetic variation. We explore two developmental models, one in which the epigenetic interactions between modules result in additive effects on character expression and a second model in which these epigenetic interactions produce nonadditive effects. Using a phenotype landscape approach, we show how changes in the developmental processes underlying phenotypic expression can alter the magnitude and pattern of quantitative genetic variation. Additive epigenetic effects influence genetic variances and covariances, but allow trait means to evolve independently of the genetic variances and covariances, so that phenotypic evolution can proceed without changing the genetic covariance structure that determines future evolutionary response. Nonadditive epigenetic effects, however, can lead to evolution of genetic variances and covariances as the mean phenotype evolves. Our model suggests that an understanding of multivariate evolution can be considerably enriched by knowledge of the mechanistic basis of character development.

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Adaptive evolution proceeds by selection acting on phenotypes, thereby altering the pattern of allelic variation in a population. The relationship between allelic and phenotypic variation is structured by ontogeny (Cheverud 1988; A. Wagner 1996). Consequently, development plays a critical role in determining how allelic variation is translated into quantitative genetic variation and covariation (Rice 2000) and, thus, how allelic variation can contribute to the evolutionary change in a trait. Although researchers stressed a central role for development in evolution for several decades (e.g., de Beer 1940; Goldschmidt 1940; Schmalhausen 1949; Waddington 1957; Wright 1968; Gould 1977; Gilbert et al. 1996), the connection between particular developmental mechanisms and the process of microevolutionary change have been examined explicitly only relatively recently (Atchley 1984; Cheverud 1984; Riska 1986; Slatkin 1987; Wagner et al. 1997; Rice 1998, 2000).

Quantitative genetic models are the primary tools that have been used to understand how selection on phenotypes translates into the genetic changes that alter phenotype distributions across generations (e.g., Lande 1979). Fundamental to these quantitative genetic models is the partitioning of phenotypic variation into various components, such as additive, dominance, and epistatic variance. This partitioning is achieved by explicitly assuming a particular model for the relationship between the observed phenotypic variation and the allelic variation that contributed to it (e.g., Cheverud and Routman 1995). There is an increasing appreciation that assumptions about the genetic architecture underlying quantitative traits can impact the usefulness of quantitative genetic models for predicting evolutionary change (Goodnight 1988; Turelli 1988; Willis and Orr 1993; Cheverud and Routman 1995). A number of analyses have also stressed the role that development can have on the quantitative genetics of traits (see Atchley 1984; Cheverud 1984; Riska 1986; Slatkin 1987; Atchley and Hall 1991; Cowley and Atchley 1992; Rice 1998, 2000), but the impact of developmental architecture on quantitative genetic parameters and on their usefulness for making evolutionary predictions is still not well established.

Here, we expand on previous advances of developmental models of quantitative genetic variation by investigating how epigenetic relationships among developmental modules transform allelic variation into quantitative genetic variation. We follow Rice's (1998, 2000) development of the phenotype landscape as a framework to illustrate how developmental processes can alter the contribution of allelic variation to additive and epistatic components of genetic variance and covariance. The joint contribution of multiple developmental modules to trait expression is visualized as a phenotype land-

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scape, where the topography of the landscape reflects the relationship between underlying developmental variables and the phenotype (for a complete description of phenotype landscapes, see Rice 1998, 2000). We use a linear model analogous to the path analytical models of Atchley et al. (Atchley 1987; Atchley and Hall 1991; Atchley et al. 1992, 1994; Cowley and Atchley 1992) to examine the specific role of developmental interactions among modules, which define the surface of our phenotype landscape.

Because of the inherent complexity of ontogeny in a real system, our model is necessarily simplistic and is not intended as a general model of trait development. Rather, our intention is to contribute to the further development of a conceptual approach that can be used to understand the influence of developmental interactions on trait evolution. We use explicit models of epigenetic interactions in trait development to illustrate how changes in developmental interactions between modules during development or changes in the underlying allelic composition of a population affect standard quantitative genetic parameters that are used to predict phenotypic evolution. Application of our model to understanding evolution in an empirical context can be achieved in cases where a particular system fits the basic assumptions of the model or in cases where one can derive appropriate predictive equations for a similar case using the general approach outlined here.

## Modularity and Epigenetics

Before exploring the effects of developmental interactions on evolutionary change, we define the developmental units of interest and the interactions that occur among them. In our model we consider interactions between modules, the developmental units that compose traits through ontogeny (see Hall 1983; Rice 2000). Most traits are a mosaic of developmental modules, with each module contributing to the trait mean, variance, and covariance with other traits. Through ontogeny, epigenetic regulatory action coordinates the development of these modules, thus further affecting the means, variances, and covariances of the characters. Evolutionary changes in a trait therefore can result from alterations in module development, epigenetic regulatory action, or both (A. Wagner 1996; G. P. Wagner 1996; Wagner and Altenberg 1996; Wagner et al. 1997). Modules are composed of tissue fields or cell types that share physical proximity and a coordinated response to epigenetic (extramodular) inductive processes or cues. Modules exist in particular locations and times through ontogeny and are dynamic—fusing, dividing, differentiating, or changing in other ways as development proceeds (Wessells 1977; Raff 1996). Modules are hierarchical in structure in that a single module is often a mosaic of smaller modules; consequently, modules can be recognized at several levels of organization such as cells, tissues, organs, or other discrete morphological traits (Wessells 1977; Hall 1992; Raff 1996). For example, the bodies of holometabolous (completely metamorphic) insects can be divided into a hierarchy of developmental modules during ontogeny. Virtually all adult structures (e.g., limbs, wings, eyes) of holometabolous insects arise from cells that are sequestered early in embryogenesis. These isolated cells undergo division and differentiation in self-contained pockets, the imaginal disks. At metamorphosis, each disk responds independently to endocrine (epigenetic) cues, everting to reveal a fully formed adult trait. Although disk development is regulated centrally by circulating hormones, there are localized effects of one disk on the ontogeny of neighboring disks. For example, experimental ablation of a wing disk increases the size of structures developing from other disks in the thoracic segment, but does not affect size of structures developing in the other segments of the insect. Similarly, the negative genetic correlation between horn and eye size in horned beetles results from interactions between imaginal disks that give rise to these structures, which share close proximity within the beetle head (Nijhout and Emlen 1998; Emlen and Nijhout 1999). These results suggest that each insect segment represents a developmental module, which in turn contains smaller interacting modules that form the adult structures. Within each disk, there are still smaller interacting modules, which eventually comprise the differentiated cell types of each adult structure. Most traits that are the focus of quantitative genetic studies are actually complex mosaics of developmental modules that interact through ontogeny to affect the value of the composite, terminal phenotype. Quantitative characters therefore can be considered to be mosaics composed of multiple modules at multiple levels of organization.

We assume that the patterns of growth and differentiation of a module are affected by intermodular, or epigenetic, interactions. The resulting epigenetic effects can be defined generally as the influence that one developing module has on the expression of other modules. Epigenetic interactions themselves can originate from the variety of modes by which a module can influence the development of other modules. Epigenetic effects can be direct inductive or regulatory interactions mediated by signaling proteins or indirect effects such as when one module modifies the local embryonic environment for neighboring modules. For example, epigenetic effects may result from competition for resources among developing modules, local embryonic morphogen gradients that affect patterns of gene expression, or physical stresses among cartilaginous structures that initiate bone deposition. These sorts of interactions can occur between physically close modules, which interact by direct physical contact, or they can occur between physically distant modules, where the interaction may occur via an intermediary (e.g., the circulatory system). Epigenetic interactions can also result from factors that influence module ontogeny by affecting the patterns of gene expression regulating the initiation, termination, and rate of module differentiation, growth, and programmed death (Wessells 1977; Hall 1983, 1992). Thus, although we will discuss epigenetic effects as a single phenomenon, they encompass a broad range of specific interactions. Furthermore, our model does not distinguish between epigenetic interactions that are temporal versus those that are spatial in nature (Hall 1992). Although we do not dwell on the distinction between these types of interactions (spatial vs. temporal), one can view our model alternatively as a model of spatial interactions, where modules exist in a spatial dimension or as a model of temporal interactions, where these modules are arrayed in a time series. We comment further on this distinction when presenting each of the models below.

## A Model for Trait Development

We now present a model of modular development based on our assumptions about the role of epigenetic effects in trait development. Using a quantitative genetic perspective, we examine how the developmental relationships among modules can affect the pattern of variation and covariation among traits and therefore the potential for evolutionary change.

Consider a single trait $i$, with phenotypic value $z_i$ that is composed of $n$ developmental modules (each denoted $M_j$, where $j$ denotes module identity) we can express the phenotypic value as:

$$z_i = \sum_{j=1}^{n} M_j . \tag{1}$$

The hierarchical nature of developmental modules allows us to decompose these modules ($M_j$) into their constituent modules ($m_q$):

$$M_j = \sum_{q=1}^{v} m_q, \tag{2}$$

where $m_q$ represents the developmental modules contributing to the value of module $M_j$. This process can continue down through the modular hierarchy (e.g., we could subdivide the $m$ modules into their modular constituents) until we reach a lower limit, e.g., the modular constituents of biochemical or genetic pathways.

We assume that the value of each module can be decomposed into intrinsic (i.e., within module) and epigenetic (i.e., among module; developmental) components (cf. Atchley and Hall 1991; Cowley and Atchley 1992). We use the term intrinsic to refer to factors that directly influence the development of a particular module and are thus inherent to that module. We divide the intrinsic component into the additive effects of genes directly contributing to the value of the module (intrinsic genetic effects) and environmental effects (intrinsic environmental effects), which may include nonadditive intrinsic genetic effects. Intrinsic genetic effects result from local gene action and arise from factors such as locally expressed enzymes, cell structure proteins (including surface proteins), and locally acting growth factors (Atchley and Hall 1991) that influence development of a given tissue field or cell lineage. Intrinsic environmental effects result from nonheritable factors that influence module development, such as temperature or resource availability. Intrinsic effects contrast with the epigenetic effects that arise when one or more modules influence the development of the focal module, $M_j$. Epigenetic effects can be additive, where each module contributes an additive influence on the development of another module, or they can be nonadditive, where the effect of one module on the development of other modules is dependent on the module's specific allelic composition (Fig. 1). The additive effect of one module ($M_j$) on the development of another module is determined by the epigenetic effect coefficient $\xi_{jk}$ (cf. Cowley and Atchley 1992). We can now express the value of a module ($M_j$) that is influenced additively by the development of other modules ($M_k$) as

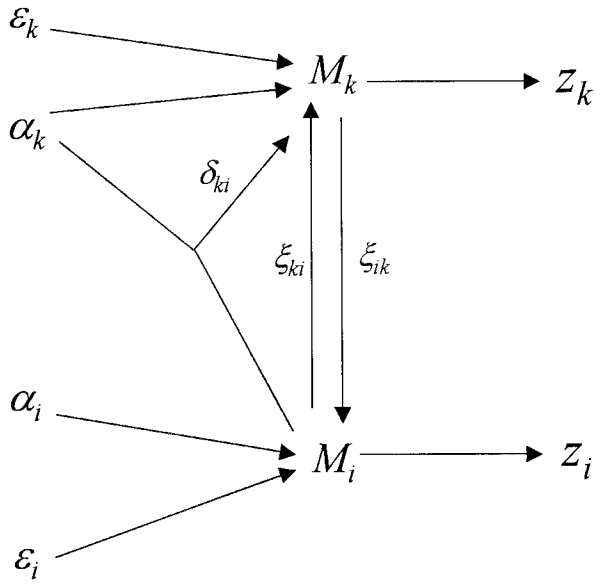


FIG. 1. Diagram of effects on the phenotype. This diagram shows the decomposition of the phenotype into modules ($M$ values), which are themselves influenced by intrinsic genetic effects ($\alpha$ values), intrinsic environmental effects ($\epsilon$ values), additive epigenetic effects ($\xi$ values), and nonadditive epigenetic effects ($\delta$ values). In this example the additive epigenetic effects are reciprocal, but the nonadditive epigenetic effects are shown as unidirectional to follow the model presented herein.

$$M_j = \alpha_j + \epsilon_j + \sum_{k=1}^{x} \xi_{jk} M_k, \tag{3}$$

where $\alpha$ denotes the intrinsic additive genetic effect and $\epsilon$ the intrinsic environmental effect.

Equation (3) can be expanded to include nonadditive epigenetic effects, where $\delta_{jk}$ is a measure of the nonadditive interaction between module $M_k$ and the intrinsic genetic component $\alpha_j$,

$$M_j = \alpha_j + \epsilon_j + \sum_{k=1}^{x} \xi_{jk} M_k + \sum_{k=1}^{x} \delta_{jk} \alpha_j M_k. \tag{4}$$

Assuming that module $M_k$ has an intrinsic genetic component contributing to its value, this nonadditive component (which represents the interaction between $\alpha_j$ and $\alpha_k$) will represent a form of physiological or developmental additive-by-additive epistasis (Crow and Kimura 1970). Figure 1 shows pathways of effects on $M_j$ corresponding to the components in equation (4).

The epigenetic effects captured in the interaction coefficients ($\xi$ and $\delta$) of equation (4) encompass a myriad of factors, including direct (e.g., inductive or regulatory interactions among modules mediated by signaling proteins) or indirect effects (Newman 1994). The interactions can be spatial or temporal, where the subscripts can designate different modules measured at a single point in time, the same module measured at different points in time, or some combination of both. As a result, this model could be used to analyze complex multidimensional interaction, time-series feedback loops, or cyclical interactions. Although we limit the analysis presented here to two simple examples to maintain clarity, we

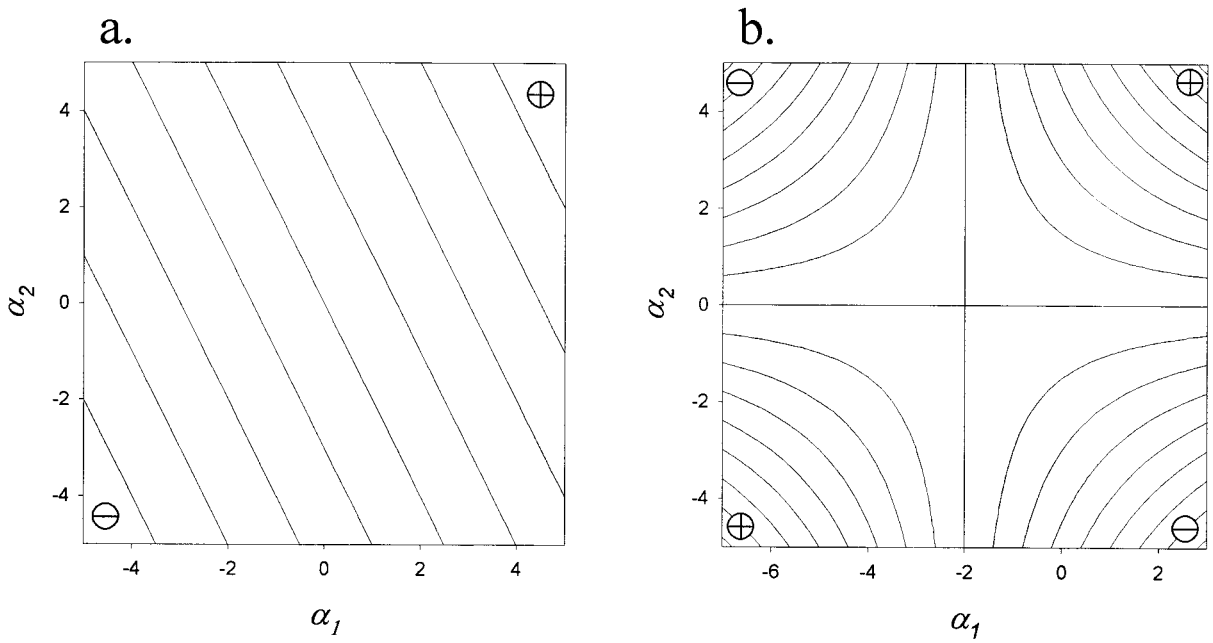


FIG. 2. Phenotype landscapes for two traits. (a) A phenotype landscape for a trait showing only additive, reciprocal, epigenetic interactions between two underlying modules. This landscape corresponds to a phenotype defined as $z_1 = M_1 = \alpha_1 + \epsilon_1 + \frac{1}{2}M_2$ (making the epigenetic effect coefficient $\xi_{12} = \frac{1}{2}$), where the phenotypic value of $M_2$ is defined as $z_2 = M_2 = \alpha_2 + \epsilon_2 + \frac{1}{2}M_1$ (see details associated with eqs. 7 and 8 in the text). (b) A phenotype landscape for a trait showing only one-way nonadditive epigenetic effects of two underlying modules. The landscape corresponds to a phenotype defined as $z_2 = M_2 = \alpha_2 + \epsilon_2 + \frac{1}{2}\alpha_2 M_1$ (where the nonadditive epigenetic effect $\delta_{21}$ has a value of $\frac{1}{2}$). $M_1$ is simply defined as the sum of intrinsic additive genetic and environmental effects $z_1 = M_1 = \alpha_1 + \epsilon_1$ (details are given for eqs. 30 and 31 in the text).

suggest that our approach could provide a fruitful way to analyze other developmental systems.

The relationship between the genotype (i.e., the intrinsic genetic effects that result from allelic composition) and the expression of the phenotype is reflected in the phenotype landscape. In the simplest case, the phenotypic value of trait $j$ ($z_j$) is equal to the sum of the values of two modules, $M_j$ and $M_k$, and the phenotype landscape of $z_j$ is a simple function of the value of intrinsic genetic effects contributing to modules $M_j$ and $M_k$. Epigenetic effects cause the landscape to take on any of a diversity of shapes depending on the values of $\xi$ and $\delta$. Figure 2 shows two hypothetical landscapes drawn as a function of the values of the intrinsic genetic components of two developmental modules underlying the trait. The first landscape (Fig. 2a) was created under the assumption of only additive epigenetic effects (i.e., $\delta_{jk} = 0$) and has the form of a plane. The second landscape (Fig. 2b) incorporates nonadditive epigenetic effects (i.e., $\delta_{jk} \neq 0$) and has the form of a saddle (Rice 1998).

### *A Two-Trait Additive Model*

We will first focus on additive epigenetic effects, where the influence of one developing module on the expression of another module is independent of the genetic or phenotypic value of that other module. We consider additive and nonadditive effects separately, but the two models could be simply combined to examine a system where both kinds of effects exist (as in eq. 4). The kinds of additive epigenetic effects considered in this first model may arise in situations such as where tissue growth is regulated by resource allocation. For example, in both butterfly (*Precis coenia*) wings and beetle (*Onthophagus taurus*) horns, the size of the structure produced appears to depend, in large part, on competition among growing parts for resources (Nijhout and Emlen 1998). Developmental regulation by gradients provides another example of where interactions may be additive. For example, the formation of wing coloration and eye-spots in butterflies depends on the concentrations of factors (including *Distilless*) around the foci that eventually form color or spots (Brakefield et al. 1996; Brakefield and French 1999).

We consider a two-trait additive model (where traits are numbered 1 and 2), where each of trait is composed of a single developmental module, $M_1$ and $M_2$, respectively (cf. eq. 1). The phenotypic value ($z$) these traits can be expressed as

$$z_1 = M_1 \quad \text{and} \tag{5}$$

$$z_2 = M_2. \tag{6}$$

Following our definition of the module from equation (3) and assuming that the two modules reciprocally affect each other during development, we can express the value of the two modules as

$$M_1 = \alpha_1 + \epsilon_1 + \xi_{12}M_2 \quad \text{and} \tag{7}$$

$$M_2 = \alpha_2 + \epsilon_2 + \xi_{21}M_1. \tag{8}$$

Unidirectional developmental effects can be considered using this general model by setting either of the epigenetic effect coefficients ($\xi_{12}$ or $\xi_{21}$) equal to zero. The reciprocal interactions shown in equations (7) and (8) can be thought of as

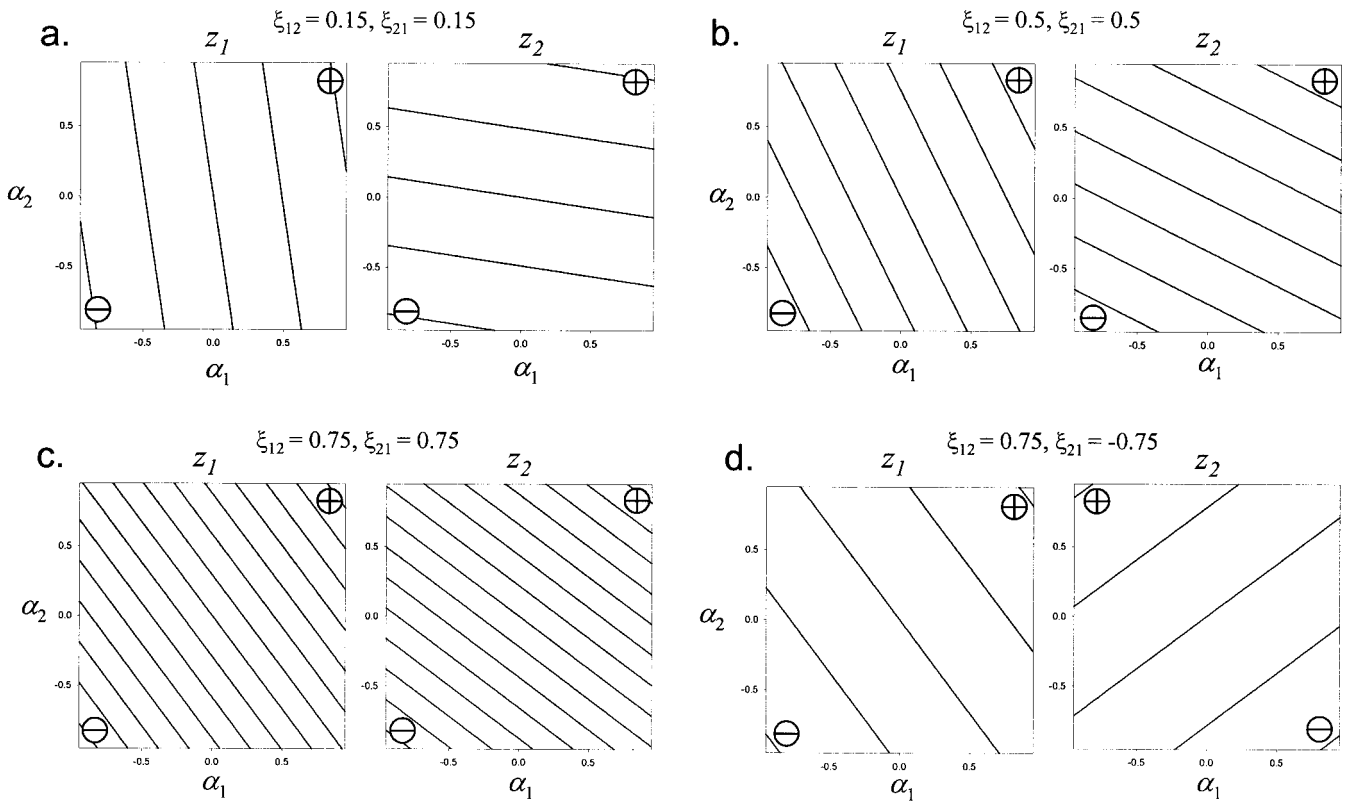


FIG. 3. Phenotype landscapes for the two-trait additive model. (a) Phenotype landscapes for traits 1 and 2 where the epigenetic effect coefficients have the values $\xi_{12} = 0.15$ and $\xi_{21} = 0.15$; (b) $\xi_{12} = 0.5$ and $\xi_{21} = 0.5$; (c) $\xi_{12} = 0.75$ and $\xi_{21} = 0.75$; (d) $\xi_{12} = 0.75$ and $\xi_{21} = -0.75$.

a model of spatial interactions because temporal interactions, by definition, are unidirectional. However, this model could be applied to temporal interactions by creating a unidirectional model as mentioned above.

Using this definition of the modules and solving for a noncircular definition of the phenotype, the phenotypic values of traits 1 and 2 are

$$z_1 = \frac{1}{1 - \xi_{12}\xi_{21}}[\alpha_1 + \epsilon_1 + \xi_{12}\alpha_2 + \xi_{12}\epsilon_2] \quad \text{and} \tag{9}$$

$$z_2 = \frac{1}{1 - \xi_{12}\xi_{21}}[\alpha_2 + \epsilon_2 + \xi_{21}\alpha_1 + \xi_{21}\epsilon_1]. \tag{10}$$

The phenotypic landscapes of $z_1$ and $z_2$, as defined by equations (9) and (10), respectively, are planes with slopes in the two dimensions ($\alpha_1$ and $\alpha_2$). The basic shape of these landscapes can be seen for various parameter values in Figure 3. The slope of the phenotype landscape for either trait can be expressed as the gradient, $\nabla z_i$. For a plane, the gradient is a vector pointing uphill on the surface in the steepest direction. The gradient vectors of the two phenotype landscapes are defined as:

$$\nabla z_1 = \left[\frac{1}{1 - \xi_{12}\xi_{21}}\right]\begin{bmatrix}1\\ \xi_{12}\end{bmatrix} \quad \text{and} \tag{11}$$

$$\nabla z_2 = \left[\frac{1}{1 - \xi_{12}\xi_{21}}\right]\begin{bmatrix}\xi_{21}\\ 1\end{bmatrix}. \tag{12}$$

To understand how this developmental view of quantitative genetic parameters corresponds with traditional quantitative genetic parameters, we can express equation (9) and (10) using the classic partitioning of the phenotype into direct additive genetic effects ($a$) and random (i.e., environmental) effects ($e$). Trait $z_1$ can be expressed as

$$z_1 = a_1 + e_1, \tag{13}$$

where $a_1$ is the additive genetic value and $e_1$ is the environmental component. These components of the phenotype are defined in our model as

$$a_1 = \frac{1}{1 - \xi_{12}\xi_{21}}[\alpha_1 + \xi_{12}\alpha_2] \quad \text{and} \tag{14}$$

$$e_1 = \frac{1}{1 - \xi_{12}\xi_{21}}[\epsilon_1 + \xi_{12}\epsilon_2]. \tag{15}$$

It follows that

$$z_2 = a_2 + e_2, \tag{16}$$

where

$$\alpha_2 = \frac{1}{1 - \xi_{12}\xi_{21}}[\alpha_1 + \xi_{21}\alpha_1] \quad \text{and} \tag{17}$$

$$e_2 = \frac{1}{1 - \xi_{12}\xi_{21}}[\epsilon_2 + \xi_{21}\epsilon_1]. \tag{18}$$

The phenotypic variances and covariances ($P_{ii}$) of the two

traits can be derived by taking the pairwise covariance ($C[z_1,z_2]$) of the trait values given in equations (9) and (10). Assuming no covariance between the genetic and environmental effects (e.g., $C[\alpha_1, \epsilon_1] = 0$) the phenotypic variance of trait $z_1$ ($P_{11}$) is

$$P_{11} = \left[\frac{1}{1 - \xi_{12}\xi_{21}}\right]^2 [V(\alpha_1) + V(\epsilon_1) + 2\xi_{12}C(\alpha_1, \alpha_2) + 2\xi_{12}C(\epsilon_1, \epsilon_2) + \xi_{12}^2V(\alpha_2) + \xi_{12}^2V(\epsilon_2)]. \quad (19)$$

Similarly the phenotypic variance of trait 2 is defined as

$$P_{22} = \left[\frac{1}{1 - \xi_{12}\xi_{21}}\right]^2 [V(\alpha_2) + V(\epsilon_2) + 2\xi_{21}C(\alpha_1, \alpha_2) + 2\xi_{21}C(\epsilon_1, \epsilon_2) + \xi_{21}^2V(\alpha_1) + \xi_{21}^2V(\epsilon_1)] \quad (20)$$

and the phenotypic covariance between the two traits is defined as

$$P_{12} = \left[\frac{1}{1 - \xi_{12}\xi_{21}}\right]^2 [C(\alpha_1, \alpha_2) + C(\epsilon_1, \epsilon_2) + \xi_{21}V(\alpha_1) + \xi_{12}V(\alpha_2) + \xi_{21}V(\epsilon_1) + \xi_{12}V(\epsilon_2) + \xi_{12}\xi_{21}C(\alpha_1, \alpha_2) + \xi_{12}\xi_{21}C(\epsilon_1, \epsilon_2)]. \quad (21)$$

The additive genetic variances ($G_{ii}$) and covariance ($G_{ij}$) can be extracted as the portion of the phenotypic variance owing to intrinsic additive genetic effects. Alternatively, the additive genetic covariances can be derived using equations (14) and (17) by taking the pairwise covariance of the additive genetic effects. Using either of these approaches, we find that the additive genetic variance of the two traits are defined as

$$G_{11} = \left[\frac{1}{1 - \xi_{12}\xi_{21}}\right]^2 [V(\alpha_1) + 2\xi_{12}C(\alpha_1, \alpha_2) + \xi_{12}^2V(\alpha_2)] \quad (22)$$

and

$$G_{22} = \left[\frac{1}{1 - \xi_{12}\xi_{21}}\right]^2 [V(\alpha_2) + 2\xi_{21}C(\alpha_1, \alpha_2) + \xi_{21}^2V(\alpha_1)], \quad (23)$$

and the additive genetic covariance between the traits is

$$G_{12} = \left[\frac{1}{1 - \xi_{12}\xi_{21}}\right]^2 [C(\alpha_1, \alpha_2) + \xi_{21}V(\alpha_1) + \xi_{12}V(\alpha_2) + \xi_{12}\xi_{21}C(\alpha_1, \alpha_2)]. \quad (24)$$

The derivation of the environmental variances ($E_{ii}$) and covariance ($E_{ij}$) is analogous to the derivation of the additive genetic covariances. Taking the covariance of the environmental terms given in equations (15) and (18), we define the environmental variances as:

$$E_{11} = \left[\frac{1}{1 - \xi_{12}\xi_{21}}\right]^2 [V(\epsilon_1) + 2\xi_{12}C(\epsilon_1, \epsilon_2) + \xi_{12}^2V(\epsilon_2)] \quad (25)$$

and

$$E_{22} = \left[\frac{1}{1 - \xi_{12}\xi_{21}}\right]^2 [V(\epsilon_2) + 2\xi_{21}C(\epsilon_1, \epsilon_2) + \xi_{21}^2V(\epsilon_1)] \quad (26)$$

and the covariance as

$$E_{12} = \left[\frac{1}{1 - \xi_{12}\xi_{21}}\right]^2 [C(\epsilon_1, \epsilon_2) + \xi_{21}V(\epsilon_1) + \xi_{12}V(\epsilon_2) + \xi_{12}\xi_{21}C(\epsilon_1, \epsilon_2)]. \quad (27)$$

### *A Two-Trait Nonadditive Model*

The occurrence of simple, additive epigenetic interactions is probably the exception rather than the rule. More common are nonadditive epigenetic interactions among modules (Wessells 1977; Hall 1992; Rice 1998, 2000). Nonadditive interactions can be reciprocal, as in feedback loops, but unlike the purely additive model, we assume that interactions are not simultaneous between interacting modules. This assumption is necessary whenever interactions are nonadditive, because covariances do not have finite dimensions when interactions occur simultaneously and reciprocally. Thus, in our model, interactions are ordered and unidirectional in nature. This is equivalent either to each trait representing new modules or to each trait representing an ontogenetic step, where $M_1$ is the phenotypic value of a module at time 1, $M_2$ is the value at time 2, $M_3$ is the value at time 3, and so on. As in the additive case, interactions can be spatial or temporal. Although the assumption of ordered interactions is necessary for mathematical tractability, it gives few limitations on the application of our model to known developmental systems, whether regulative or mosaic development (see Discussion). Even reciprocal interactions can be decomposed into multiple steps where effects are unidirectional. Both instructive and permissive interactions involve sequential interactions, even when developmental feedback is involved.

For simplicity, we first present a model of nonadditive interactions reflecting interactions between two modules at the first step. To accommodate interactions that occur beyond the first step, our model can be extended to multiple sequences of interactions. In the appendix, we extend sequences to a third time step to show how an extended developmental series will influence quantitative genetic parameters. Looped feedback systems can be analyzed by assuming that the module represented as $M_3$ is the same module as $M_1$, but is measured at the end of a feedback loop (whereas $M_1$ is the value at the start of the loop).

As above, we examine two traits, each made up of a single module:

$$z_1 = M_1 \quad \text{and} \quad (28)$$

$$z_2 = M_2. \quad (29)$$

We assume that the traits represent sequential steps in ontogeny, where $z_1$ is the phenotypic value of a character at step 1, $z_2$ is the phenotypic value at step 2, and so on. We define the value of the first module (contributing to trait $z_1$) as:

$$M_1 = \alpha_1 + \epsilon_1. \quad (30)$$

In equation (30), we have assumed that at the first time step

in the series there are no epigenetic interactions. This is the initiation of ontogeny, where we assume that only intrinsic genetic and environmental factors influence the developmental process, independent of any previous developmental events. More information may be gained if the state of various modules at previous time steps are known, but to examine the effects of interactions through ontogeny we can arbitrarily assign any point in ontogeny as the initial state and examine interactions among modules forward from that point. The value of this same trait at the next step in the developmental series will be influenced by the value at time step 1 ($M_1$):

$$M_2 = \alpha_2 + \epsilon_2 + \delta_{21}\alpha_2 M_1. \quad (31)$$

The term $\delta_{21}\alpha_2 M_1$ describes the simplest nonadditive epigenetic effect of one module, $M_1$, on a second, $M_2$. This term describes an effect of $M_1$ on $M_2$ that depends not only on the value of $M_1$ (as in the additive model, see eq. 3) but also on the genes expressed in $M_2$. $\delta_{21}$ is a coefficient that determines the magnitude of the epigenetic effect and is analogous to the coefficient $\xi$ in the additive model. By incorporating the definition of module 1 into the value of module 2, equation (31) can be written as:

$$M_2 = \alpha_2 + \epsilon_2 + \delta_{21}\alpha_2\alpha_1 + \delta_{21}\alpha_2\epsilon_1. \quad (32)$$

We can interpret the components contributing to the expression of $M_2$ by examining the components in equation (32). The term $\delta_{21}\alpha_2\alpha_1$ describes an interaction between the intrinsic genetic components in $M_1$ and $M_2$, which corresponds to additive-by-additive epistasis because the two intrinsic components are, by definition, additive in their direct effects on each module. The term $\delta_{21}\alpha_2\epsilon_1$ describes an interaction between the genetic component in $M_2$ and environmental effects influencing $M_1$, which corresponds to a genotype-by-environment (G × E) interaction effect. The effect that the intrinsic effect $a_2$ has on the expression of $M_2$ depends on the environmental effect on $M_1$. The phenotype landscape for trait 2 is shown in Figure 2b. It is important to note that the equations presented above that define the trait values (eqs. 28–32) do not represent a statistical model, but rather a developmental model of interacting modules (as in Rice 1998).

As with the additive model, we can use the developmental model (eqs. 31, 32) to describe the phenotypic covariances as a function of variation in the underlying components. For mathematical tractability, we make three assumptions common in quantitative genetic models (Lynch and Walsh 1998): (1) the underlying genetic and environmental components are multivariate normally distributed; (2) the expected environmental deviations are zero, $E(\delta_i) = 0$; and (3) there is no covariance between genetic and environmental effects, $C(\alpha_i,\delta_i) = 0$. To avoid redundancy, we present only the genetic and environmental variances and covariances, noting that the phenotypic variances and covariances are simply the sum of the appropriate genetic and environmental components (see examples in the additive model).

The additive genetic variances of the two traits are:

$$G_{11} = V(\alpha_1) \quad \text{and} \quad (33)$$

$$G_{22} = V(\alpha_2) + \delta_{21}^2[\bar{\alpha}_2^2 V(\alpha_1) + \bar{\alpha}_1^2 V(\alpha_2) + 2\bar{\alpha}_1\bar{\alpha}_2 C(\alpha_1, \alpha_2)] + 2\delta_{21}[\bar{\alpha}_2 C(\alpha_1, \alpha_2) + \bar{\alpha}_1 V(\alpha_2)]. \quad (34)$$

The additive genetic covariance is defined as:

$$G_{12} = C(\alpha_1, \alpha_2) + \delta_{21}\,\bar{\alpha}_2 V(\alpha_1). \quad (35)$$

This equation shows that the additive genetic covariance between the two traits depends on the covariance between the intrinsic genetic effects in each module and the covariance between the intrinsic genetic effect in $M_1$ with the epigenetic effect in $M_2$, i.e., $C(\alpha_1, \delta_{21}\,\alpha_2\alpha_1)$.

Because our developmental model is nonadditive, the potential exists for interaction (i.e., epistatic) components of genetic variance. However, because our developmental model does not allow for interactions in the development of trait 1, the additive-by-additive epistatic variance of $z_1$ ($I_{11}$) is by definition zero. Similarly, the epistatic covariance between traits 1 and 2 ($I_{12}$) is also zero by definition. The epistatic variance of trait 2 is defined as:

$$I_{22} = \delta_{21}^2[V(\alpha_1)V(\alpha_2) + C^2(\alpha_1, \alpha_2)] = \delta_{21}^2(1 + \rho_{12}^2)V(\alpha_1)V(\alpha_2), \quad (36)$$

where $\rho_2$ is the correlation between $\alpha_1$ and $\alpha_2$. From equation (36) we can see that the contribution of allelic variance to epistatic variance depends on the square of the epigenetic effect coefficient, $\delta_{21}$, and the square of the correlation between the genetic components, $\rho_{12}$.

We define the environmental variances as:

$$E_{11} = V(\epsilon_1) \quad (37)$$

$$E_{22} = V(\epsilon_2) + (\delta_{21}\bar{\alpha}_2)^2 V(\epsilon_1) + 2\delta_{21}\bar{\alpha}_2 C(\epsilon_1, \epsilon_2), \quad (38)$$

and

$$E_{12} = C(\epsilon_1, \epsilon_2) + \delta_{21}\bar{\alpha}_2 V(\epsilon_1). \quad (39)$$

Note that the environmental variance terms in equations (38) and (39) resulting from the epigenetic effect depend on the mean value of the genetic component in $M_2$ ($\alpha_2$) because of the developmental G × E interaction. This term shows the potential for evolution of canalization of the phenotype in a variable environment (Wagner et al. 1997; Rice 1998, 2000).

Because of the nonadditive developmental interaction term in equation (31), there is the potential for G × E variance, $V(G \times E)_{ii}$. As in the case of the epistatic variance, the G × E variance of trait 1 ($V[G \times E]_{11}$) is zero because there are no developmental interactions affecting the expression of $z_1$. For this same reason, the G × E covariance between $z_1$ and $z_2$ ($V[G \times E]_{12}$) is also zero. We define this component of variance for the traits as:

$$V(G \times E)_{22} = \delta_{21}^2\, V(\epsilon_1)V(\alpha_2). \quad (40)$$

From equation (40) we see that the G × E variance depends the variance for environmental effects on $M_1$ and variance for genetic effects in $M_2$. As with additive-by-additive genetic variance components (eq. 36), the G × E variance component depends on the square of the epigenetic coefficient, $\delta_{21}$, and is large only when the epigenetic effect is large.

## Discussion

Quantitative genetic theory provides a framework for understanding the dynamics of evolutionary change in a trait without requiring knowledge of its underlying genetic and

developmental architecture. However, the very assumptions of quantitative genetics that allow inferences to be made about the structure of genetic variation that underlies phenotypic variation (see Falconer and Mackay 1996) may obscure interesting and potentially important aspects of biology that will affect the evolution of populations. By adopting a modular view of trait development, we have explicitly considered how interactions among modules influence the pattern of phenotypic and genetic variation in characters. Most traits are mosaics of developmental modules, with each module contributing additively or nonadditively to a trait's phenotypic value. The kinds of interactions that underlie module ontogeny influence both the phenotypic and quantitative genetic variation of traits, as well as the correlations among them. This pattern of variation and covariation determines the potential evolutionary responses of traits affected by selection and therefore connects the developmental process to quantitative genetic models of evolutionary change. Inclusion of the developmental basis of trait ontogeny may refine our understanding of trait evolution and may indicate that, in the absence of consideration of trait ontogeny, assumptions of quantitative genetic approaches may sometimes lead to a misunderstanding of the quantitative genetic variation of traits and inaccurate predictions about trait evolution within populations.

Explicit consideration of the developmental interactions that underlie phenotypic expression generates a number of predictions regarding the relationships between the strength and form of epigenetic interactions and quantitative genetic parameters. These predictions inform us about how changes (experimental or evolutionary) in the developmental program or in the allelic composition of populations can impact quantitative genetic parameters. In general, we find that critical quantitative genetic parameters, like the additive genetic variance or covariance of traits, may be sensitive to changes in the regulation of development that translates allelic variation into phenotypic variation. In addition, we find that nonadditive epigenetic effects can tie the evolution of genetic variances and covariances to the phenotypic mean, thus complicating our ability to predict the direction of evolutionary change across many generations without knowledge of developmental architecture.

These results can be understood by examining the phenotypic landscapes that are generated by our model. In the sections that follow, we discuss how to interpret phenotype landscapes and then explain how different types of interactions between modules generate different shapes of the phenotype landscape. We first explore simple linear landscapes before moving on to more complex surfaces, showing how even a simplistic appreciation of the mechanistic basis of quantitative traits can improve our understanding of the sources and evolutionary lability of quantitative genetic variation.

### *Interpretation of the Phenotype Landscape*

The phenotype landscape is a visualization of how some underlying factors contribute to phenotypic values (Rice 1998, 2000; see Fig. 2). These underlying factors can be any phenotypic components such as the value of underlying modules, the intrinsic genetic value of these modules, or environmental effects. We view the phenotype landscapes presented here as surfaces of values that are functions of the intrinsic genetic components of modules (Fig. 2). On the phenotype landscape, a population exists as a distribution of phenotypes determined by the current allelic variation of the population. At any given time, the population only experiences the region of the landscape covered by this distribution. The average slope and curvature of the landscape in this particular region determine the quantitative genetic parameters of the population (Rice 2000). The slope of the surface determines the phenotypic variance as a function of the underlying modules; higher variance is associated with steeper slopes. Thus, the additive genetic variance produced by a given amount of variance in an underlying module corresponds directly to the slope of the surface along that axis. When the slope is not constant across the landscape (i.e., when the landscape is curved), the position of the population on the landscape will determine the average slope experienced by a population (Rice 2000). Additive genetic variances therefore depend on the location of the population in genetic space (i.e., the mean values of the intrinsic genetic effects), as well as the form of developmental interactions among modules (i.e., the magnitude and sign of epigenetic effects) contributing to complex traits.

Phenotype landscapes can also be used to help us understand covariances between traits. Multiple traits can be expressed as separate landscapes that are functions of the same developmental modules (see Fig. 3). Genetic covariances are positive if the gradients are of the same sign for both landscapes and negative when the gradients oppose. The genetic covariance is zero only when the gradients of the landscapes are at 90° angles to one another (see Fig. 3d). When the gradients are at right angles the population can move along a contour on one landscape while moving uphill or downhill on the other landscape (see Fig. 3d). Thus, when the gradients of two landscapes are at right angles the two characters can evolve independently.

### *Linear Landscapes and Additive Effects*

Intrinsic genetic effects on different modules can combine to influence a single phenotype through epigenetic effects. When all epigenetic effects between modules are additive, they result in a planar landscape (Rice 1998, 2000) with a gradient determined by the epigenetic effect coefficients, $\xi_{12}$ and $\xi_{21}$ (see eqs. 11 and 12). Thus, in this case, intrinsic genetic effects contribute additive genetic variance in proportion to their additive contribution to the phenotypic value (as measured by $\xi_{12}$ and $\xi_{21}$, see eqs. 22 and 23). Because slopes are constant across the landscape, a module's effect on additive variance is the same regardless of the location of the population on the landscape. Thus, changes in the mean can occur independently of changes in the additive genetic variance, thereby allowing phenotypic evolution to proceed without changing the genetic variances that determine future evolutionary response (Lande 1979; Turelli 1988). However, factors that lead to changes in the gradients of the phenotype landscapes (i.e., changes in either $\nabla z_1$ or $\nabla z_2$) will result in changes in the additive genetic variances (see Fig. 4a and

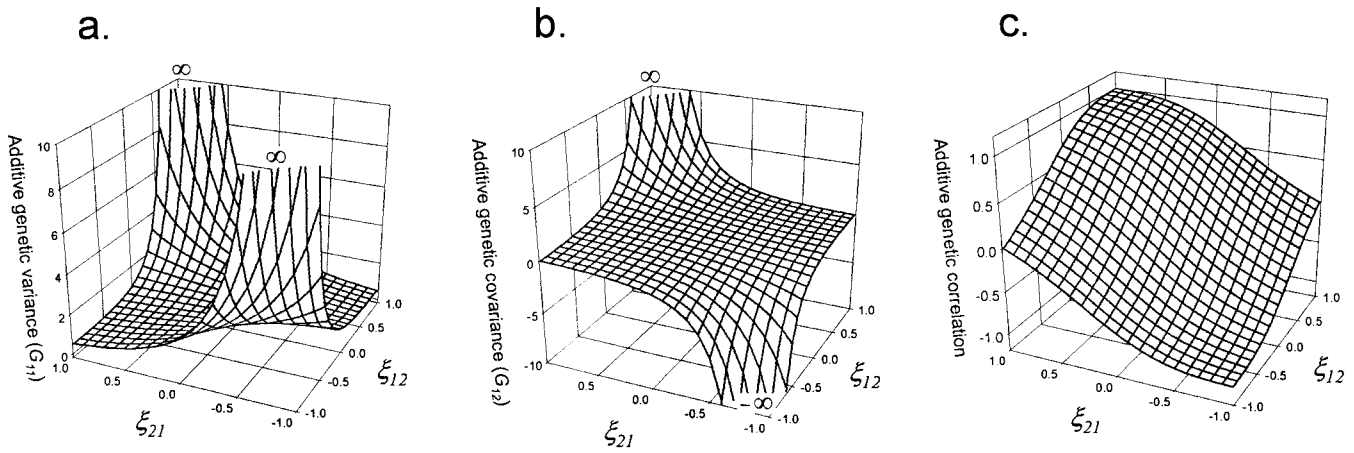


FIG. 4. Quantitative genetic variation as a function of the additive epigenetic effect coefficients ($\xi_{12}$ and $\xi_{21}$). (a) The additive genetic variance ($G_{11}$ or $G_{22}$) as a function of the values of the additive epigenetic effect coefficients (this single figure gives the additive genetic variance of either character). This figure was drawn using equation (20) (eq. 21 for $G_{22}$). The region of the surface near the (1,1) and (−1, −1) corners of the surface have been truncated to display a finer scale in the figure. These regions of the surface approach a phenotypic value of infinity and are indicated in the figure by an ∞ symbol. (b) The additive genetic covariance ($G_{12}$) between the two traits as a function of the additive epigenetic effect coefficients. This figure was created using equation (24) assuming that there is no covariance between the intrinsic genetic effects. The region of the surface approaching (1,1) and (−1, −1) have been truncated because they approach phenotypic values of $+\infty$ and $-\infty$, respectively. (c) The genetic correlation between the two traits as a function of the additive epigenetic effect coefficients (calculated using the data from Figs. 4a, b).

eqs. 22 and 23). Because the gradients of the two landscapes do not change linearly with changes in the epigenetic effect coefficients, the additive genetic variances also do not change linearly with changes in the strength of epigenetic interactions (eqs. 22 and 23). Rather, the slopes approach infinity as the value of the product of the epigenetic effect coefficients approaches one. This can be attributed to the feedback loop between the two modules that regulates the development of the two characters. This feedback loop can amplify variation in the traits and therefore can lead to dramatic changes in genetic variances when the strength of epigenetic interactions changes.

Intrinsic genetic covariation between modules contributes to the additive genetic covariance between traits because it indicates that either allelic effects are contributing to multiple modules (i.e., ''intrinsic pleiotropy'' sensu Atchley and Hall 1991) or there is linkage disequilibrium between loci influencing the two characters. Beyond the contribution of this intrinsic genetic covariation between modules, we find that epigenetic interactions can produce genetic covariation between traits. Because of this, small changes in the value of the additive epigenetic coefficient (i.e., either $\xi_{12}$ or $\xi_{21}$) can lead to relatively large changes in the additive genetic covariance without any changes in the intrinsic genetic variance or covariance (Fig. 4b). In this way, epigenetic effects can modify the underlying developmental covariation between traits. Whether the epigenetic effects increase or decrease the additive genetic covariance depends on the sign of the effect and the sign of the underlying intrinsic covariance (Fig. 4b). As in the case of the additive genetic variance on planar phenotypic landscapes, the genetic covariance will not change with evolution of the mean phenotype because the slopes are the same regardless of the location of population in genetic space.

The effect of changing the strength of epigenetic interactions on the quantitative genetics of a population can be visualized using Figure 3, which shows four pairs of phenotype landscapes corresponding to four different values for the two epigenetic effect coefficients (i.e., strengths of developmental interactions). The first three examples (Figs. 3a, b, c) illustrate the effect of increasing the strength of epigenetic interaction while keeping the signs of the interaction effects the same. We can begin by examining Figure 3a, where the two epigenetic effect coefficients are relatively small (both have a value of 0.15). The landscapes are relatively flat, which is reflected in the small additive genetic variance, $G_{ii} = 1.07$ for both traits (calculated assuming that both intrinsic genetic variance have a value of 1.0). In this case we also see that the two landscapes are nearly at right angles to one another (i.e., the contour lines are nearly perpendicular), which results in a small genetic covariance, $G_{12} = 0.314$ (calculated assuming no intrinsic genetic covariance and again assuming intrinsic genetic variances of 1.0). Figures 3b and 3c illustrate the impact of increasing the strength of epigenetic interactions. First, we see that the landscapes become steeper, which is reflected in the additive genetic variances of the two traits in these two cases, which are 2.222 and 8.163 for the parameter values in Figures 3b and 3c, respectively (calculated under the same assumptions as above). From Figures 3a, b, and c we can also see that the landscapes rotate and become aligned (i.e., the gradient vectors converge) as the interaction effect becomes stronger, which results in larger additive genetic covariances in these two examples (where $G_{12} = 1.778$ for the case in Fig. 3b and $G_{12} = 7.837$ for the case in Fig. 3c and under the assumptions stated above).

In Figure 3d we see a case where the epigenetic effects oppose one another (i.e., are of opposite sign). In this case, the two landscapes are relatively flat because the feedback loop between the modules during development acts to damp-

en the impact of genetic variation on phenotypic variation of the two traits. This results in a small additive genetic variance (where $G_{11} = G_{22} = 0.64$ under the assumptions given above). Also, the opposing epigenetic effects place the two landscapes at right angles in this particular case, which results in a zero genetic covariance despite the fact that the two sets of intrinsic genetic effects contribute to the expression of both characters. This outcome can be visualized using Figure 3d, where one can see that sliding along a contour line on the landscape of $z_1$ (which, by definition, results in no change in the mean of $z_1$) results in an uphill or downhill movement on the landscape of $z_2$.

Because epigenetic effects alter both genetic variances and covariances, it may be more informative to examine the genetic correlation between traits 1 and 2 as a function of the epigenetic effect coefficients (Fig. 4c). We find a simple relationship between epigenetic effects and the genetic correlation: When both effects are negative, the genetic correlation is negative, and when both are positive, the correlation is positive. When the two coefficients are of opposite sign, the sign of the genetic correlation is determined by the relative magnitude of the two coefficients.

Developmental interactions do not affect trait heritability because they influence both the environmental and additive genetic variances in a similar way (see eqs. 22, 23, 25, 26). Developmental interactions change the environmental variance at the same rate that they change the additive genetic variance so the heritability ratio remains constant across all values of epigenetic effect coefficients ($\xi_{12}$ or $\xi_{21}$). Nonetheless, additive epigenetic interactions can affect the response of a trait to selection in two ways. First, such interactions can inflate additive genetic variance even though the ratio of additive genetic to phenotypic variance, i.e., the heritability, does not change. Such changes thereby alter the ''evolvability'' (sensu Houle 1992) of traits (i.e., their evolutionary lability). Second, the value of the genetic covariance as a function of the epigenetic effect coefficients ($\xi_{12}$ or $\xi_{21}$) does not change at the same rate as the additive genetic variance (see eqs. 22–24, Fig. 4). Therefore, changes in developmental interactions between modules can alter additive genetic correlations (Fig. 4c) and consequently affect correlated responses to selection and multivariate evolution.

### *Nonlinear Landscapes and the Evolution of Genetic Variation*

The most important consequence of nonadditive interactions between modules (i.e., $\delta_{ij}$) during development is to generate curvature of the phenotype landscape (Rice 1998, 2000). This curvature results in a variable slope across the phenotype landscape and therefore genetic parameters depend on the location of the population on the landscape. The result is that the evolution of phenotypic means is correlated with the evolution of genetic variances and covariances when nonadditive epigenetic effects exist. Resulting changes in the additive genetic variance covariance structure (usually represented as a matrix of variances and covariances, i.e., the **G**-matrix) alter the rate and trajectory over the course of phenotypic evolution (Lande 1979; Turelli 1988).

In our model, nonadditive effects are only unidirectional

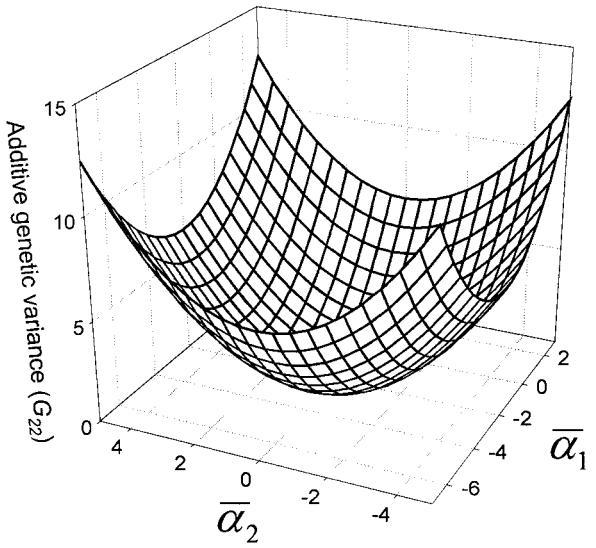


FIG. 5. Additive genetic variance as a function of the mean value of the intrinsic genetic effects contributing to the phenotype for the nonadditive epigenetics case. The variance was calculated assuming that the variance of both intrinsic genetic effects ($V[\alpha_1]$ and $V[\alpha_1]$) are equal (having the value one). The variance was calculated using equation (34) with a nonadditive epigenetic effect coefficient set at a value of 0.5.

and consequently can be considered as steps through ontogeny between interacting modules. The expression of the first trait in the sequence ($z_1$) depends only on the value of the intrinsic genetic effect contributing to that trait ($\alpha_1$), so the phenotypic landscape of this trait is a simple plane that slopes only on the axis of $\alpha_1$. The second trait, developing later in ontogeny, is influenced by nonadditive interactions between the developmental modules (see eq. 31), resulting in a curved phenotypic landscape with a slope that changes at a constant rate as a function of the mean of each character (Fig. 2b). As a result, additive genetic variance changes as a population moves on the landscape (Fig. 5), increasing as the population moves away from the saddle point on the phenotypic landscape (Fig. 2b). Similarly, the additive genetic covariance between the traits increases linearly as a function of the mean value for the intrinsic effects contributing to the traits at a rate determined by the nonadditive epigenetic effect coefficient (eq. 35). Because of this relationship between the mean and covariance structure, the additive genetic variance can increase or decrease depending on the direction of evolution and the curvature of the landscape. For example, if a population starts close to the saddle point on the landscape in Figure 2b and selection drives it toward one of the corners, the additive variance will increase during the course of selection, accelerating the rate of evolutionary response to selection. Similar results should be found in other mechanistic models of quantitative variation (e.g., Cheverud and Routman 1995), where physiological interactions between loci make the additive variance evolve as selection drives a population through a range of gene frequencies.

Nonadditive epigenetic effects can link the evolution of the mean to the evolution of covariance structure. At the same time, they may also result in situations where variances can

evolve while phenotypic means remain constant. Because of this, two populations with the same variances in underlying components (i.e., intrinsic genetic or environmental variances) can produce the same trait mean but with different phenotypic variances. Thus, populations can differentiate in their genetic variances by sliding along contours of equal phenotypic value, without experiencing correlated changes in the mean value of any traits (Rice 2000). This sort of evolution of genetic variance has been considered an important component in the evolution of genetic canalization (Rice 1998) and is expected to occur when stabilizing selection is applied to a population. This can easily be examined by combining our model with a simple model of stabilizing selection.

The nonadditive epigenetic interactions also provide the opportunity for G × E interactions to occur. Because the nonadditive epigenetic effect in our model occurs when the phenotypic value of module 1 interacts with the genetic value for module 2, the environmental effects on module 1 (i.e., intrinsic environmental effects) can interact with the genetic effects on module 2 (eq. 32). In this case, the G × E variance is the same, regardless of the location of the population on the landscape, and changes as a function of the square of the nonadditive epigenetic effect coefficient. Because of the unidirectional interaction, the other G × E variance and covariance have a value of zero. In both the additive and the nonadditive model, we see that there can be environmental covariances between traits even in the absence of intrinsic environmental covariances. This relationship occurs because the epigenetic effect transforms environmental effects on one character into environmental effects on the other character, thus producing environmental covariation between the traits.

Epistatic genetic variance is proportional to the curvature of the phenotype landscape (eq. 38). In our two-trait model, curvature is constant across the landscape. Evolutionary changes in the mean phenotypes therefore do not lead to changes in the amount of epistatic variance. It is also clear from equation (36) that only extreme values of nonadditive epigenetic effects (and consequently extreme curvature of the phenotype landscape) will lead to high values of epistatic genetic variance. In fact, most of the nonadditive interaction between intrinsic effects is manifest as additive genetic variance (see also Cheverud and Routman 1995).

Development obviously involves numerous steps and interactions beyond the two-module scenario presented above. We have restricted our model to the simple case to facilitate a heuristic understanding of the role of development in evolution, but our approach can be easily extended to examine more complex patterns of interaction. The inclusion of additional interactions, however, dramatically increases the number of factors that influence genetic covariance structure. In the appendix, we present a model that adds a third time step (which could also be viewed as a third interacting module) to the nonadditive model and show that factors that have purely additive effects in earlier time steps contribute nonadditive variance in later time steps. This result implies that selection may be more efficient earlier in development when the constituents of genetic variance are more likely to be purely additive. We also find that the addition of a third time step creates a situation where the epistatic and G × E variances change as a function of the location of the population on the phenotype landscape. This interdependence occurs because the landscape for the third time step does not have a constant curvature. Thus, we see that when development becomes more complex than the simple two-trait interaction modeled above, all components of quantitative genetic variation can change as a population evolves across the phenotype landscape.

### *Conclusions*

Quantitative genetic theory provides a framework for understanding the dynamics of evolutionary change in a trait without requiring knowledge of its underlying molecular genetic and developmental architecture. However, the value of quantitative genetic models for predicting evolutionary change is largely determined by the accuracy of the assumptions upon which they are built. We show how even a simplistic appreciation of the developmental basis of quantitative traits can improve our understanding of the sources and evolutionary lability of quantitative genetic variation. Our model, in combination with other recent models analyzing the constituents of quantitative genetic variation (e.g., Cowley and Atchley 1992; Cheverud and Routman 1995; Rice 1998, 2000) demonstrates that not all sources of quantitative genetic variation are equivalent with respect to how they impact phenotypic evolution over many generations. More empirical studies are needed to determine the value of considering trait development in quantitative genetic models of evolution. To be most informative, such studies should combine quantitative genetic studies with explorations and manipulations of the proximate basis of trait ontogeny. These sorts of studies have already begun to build our understanding of modular interactions (e.g., Nijhout and Paulsen 1997; Klingenberg and Nijhout 1998; Nijhout and Emlen 1998; Emlen and Nijhout 1999) and how the developmental system responds to selection on phenotypes (e.g., Brakefield et al. 1996). We should continue to build on these efforts to fully elucidate how developmental interactions affect the structure of quantitative genetic variation and the evolutionary trajectories of populations.

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## Appendix

In the text we analyzed the first two steps in a developmental series. Here we add a third time step to the model to examine how quantitative genetic parameters change as a function of the number of steps in the developmental sequence. The definition of the value of the third module in the series ($M_3$) is analogous to the definition of the module at time 2 ($M_2$) presented in equation (31):

$$M_3 = \alpha_3 + \epsilon_3 + \delta_{32}\alpha_3 M_2. \quad \text{(A1)}$$

We can substitute the definition of $M_2$ given in equation (32), which yields:

$$M_3 = \alpha_3 + \epsilon_3 + \delta_{32}\alpha_3\alpha_2 + \delta_{32}\alpha_3\epsilon_2 + \delta_{32}\delta_{21}\alpha_3\alpha_2\alpha_1 + \delta_{32}\delta_{21}\alpha_3\alpha_2\epsilon_1. \quad \text{(A2)}$$

The first three terms in equation (A2) are analogous to the terms in equation (32). The last two terms do not appear at time step 2, and represent higher-order interactions that can only occur further down the developmental sequence. The first of these terms is a three-way epistatic interaction that corresponds to additive-by-additive-by-additive epistasis. The last term is a G × E interaction term. This last term can be viewed as an epistasis-by-environmental interaction, where the component of two-way epistasis between $\alpha_2$ and $\alpha_3$ is dependent upon the environmental effect (and vice versa).

As in the main model, the total phenotypic variance of $M_3$ and covariance between $M_3$ and the other modules is simply the sum of the appropriate components of genetic and environmental variance and covariance. In the three-trait system, we define three covariances. The first of these covariances, between $M_1$ and $M_2$, is

given in the text. The variance of $M_3$ and the remaining two covariances can be derived as above and are defined as:

$$P_{33} = V(M_3) = G_{33} + I_{33} + J_{33} + E_{33} + V(G \times E)_{33}, \quad \text{(A3)}$$

$$P_{13} = C(M_1, M_3) = G_{13} + I_{13} + J_{13} + E_{13} + V(G \times E)_{13}, \quad \text{(A4)}$$

and

$$P_{23} = C(M_2, M_3) = G_{23} + I_{23} + J_{23} + E_{23} + V(G \times E)_{23}, \quad \text{(A5)}$$

where the parameters $G_{ij}$, $I_{ij}$, $E_{ij}$, and $V(G \times E)_{ij}$ are as defined in the text, and the parameter $J_{ij}$ is a three-way epistatic interaction (additive-by-additive-by-additive).

Following the derivation presented in the text, the additive genetic variance and covariances involving $M_3$ are defined as:

$$\begin{aligned} G_{33} = {} & V(\alpha_3) + \delta_{32}^2[\bar{\alpha}_3^2 V(\alpha_2) + \bar{\alpha}_2^2 V(\alpha_3) + 2\bar{\alpha}_3\bar{\alpha}_2 C(\alpha_2, \alpha_3)] \\ & + \delta_{32}^2\delta_{21}^2[\bar{\alpha}_2^2\bar{\alpha}_3^2 V(\alpha_1) + \bar{\alpha}_1^2\bar{\alpha}_3^2 V(\alpha_2) + \bar{\alpha}_1^2\bar{\alpha}_2^2 V(\alpha_3) \\ & \quad + 2\bar{\alpha}_1^2\bar{\alpha}_2\bar{\alpha}_3 C(\alpha_2, \alpha_3) + 2\bar{\alpha}_1\bar{\alpha}_2^2\bar{\alpha}_3 C(\alpha_1, \alpha_3) \\ & \quad + 2\bar{\alpha}_1\bar{\alpha}_2\bar{\alpha}_3^2 C(\alpha_1, \alpha_2)] \\ & + 2\delta_{32}[\bar{\alpha}_2 V(\alpha_3) + \bar{\alpha}_3 C(\alpha_2, \alpha_3)] \\ & + 2\delta_{32}\delta_{21}[\bar{\alpha}_1\bar{\alpha}_2 V(\alpha_3) + \bar{\alpha}_1\bar{\alpha}_3 C(\alpha_2, \alpha_3) + \bar{\alpha}_2\bar{\alpha}_3 C(\alpha_1, \alpha_3)] \\ & + 2\delta_{32}^2\delta_{21}[\bar{\alpha}_1\bar{\alpha}_2^2 V(\alpha_3) + \bar{\alpha}_1\bar{\alpha}_3^2 V(\alpha_2) + \bar{\alpha}_2^2\bar{\alpha}_3 C(\alpha_1, \alpha_3) \\ & \quad + \bar{\alpha}_2\bar{\alpha}_3^2 C(\alpha_1, \alpha_2) + 2\bar{\alpha}_1\bar{\alpha}_2\bar{\alpha}_3 C(\alpha_2, \alpha_3)], \end{aligned} \quad \text{(A6)}$$

$$\begin{aligned} G_{13} = {} & C(\alpha_1, \alpha_3) + \delta_{32}[\bar{\alpha}_3 C(\alpha_1, \alpha_2) + \bar{\alpha}_2 C(\alpha_1, \alpha_3)] \\ & + \delta_{32}\delta_{21}[\bar{\alpha}_2\bar{\alpha}_3 V(\alpha_1) + \bar{\alpha}_1\bar{\alpha}_2 C(\alpha_1, \alpha_3) + \bar{\alpha}_1\bar{\alpha}_3 C(\alpha_1, \alpha_2), \end{aligned} \quad \text{(A7)}$$

and

$$\begin{aligned} G_{23} = {} & C(\alpha_2, \alpha_3) + \delta_{32}[\bar{\alpha}_3 V(\alpha_2) + \bar{\alpha}_2 C(\alpha_2, \alpha_3)] \\ & + \delta_{32}\delta_{21}[\bar{\alpha}_1\bar{\alpha}_3 V(\alpha_2) + \bar{\alpha}_1\bar{\alpha}_2 C(\alpha_2, \alpha_3) + \bar{\alpha}_2\bar{\alpha}_3 C(\alpha_1, \alpha_2)] \\ & + \delta_{21}[\bar{\alpha}_1 C(\alpha_2, \alpha_3) + \bar{\alpha}_2 C(\alpha_1, \alpha_3)] \\ & + \delta_{32}\delta_{21}[\bar{\alpha}_1\bar{\alpha}_2 C(\alpha_2, \alpha_3) + \bar{\alpha}_1\bar{\alpha}_3 V(\alpha_2) + \bar{\alpha}_2^2 C(\alpha_1, \alpha_3) \\ & \quad + \bar{\alpha}_2\bar{\alpha}_3 C(\alpha_1, \alpha_2)] \\ & + \delta_{32}\delta_{21}^2[\bar{\alpha}_2^2\bar{\alpha}_3 V(\alpha_1) + \bar{\alpha}_1^2\bar{\alpha}_3 V(\alpha_2) + \bar{\alpha}_1\bar{\alpha}_2^2 C(\alpha_1, \alpha_3) \\ & \quad + \bar{\alpha}_1^2\bar{\alpha}_2 C(\alpha_2, \alpha_3) \\ & + 2\bar{\alpha}_1\bar{\alpha}_2\bar{\alpha}_3 C(\alpha_1, \alpha_2)]. \end{aligned} \quad \text{(A8)}$$

The two-way (additive-by-additive) epistatic genetic variance and covariances are defined as:

$$\begin{aligned} I_{33} = {} & \delta_{32}^2[V(\alpha_2)V(\alpha_3) + C^2(\alpha_2, \alpha_3)] \\ & + \delta_{32}^2\delta_{21}^2[4\bar{\alpha}_2\bar{\alpha}_3[V(\alpha_1)C(\alpha_2, \alpha_3) + 2C(\alpha_1, \alpha_2)C(\alpha_1, \alpha_3)] \\ & \quad + 4\bar{\alpha}_1\bar{\alpha}_3[V(\alpha_2)C(\alpha_1, \alpha_3) + 2C(\alpha_1, \alpha_2)C(\alpha_2, \alpha_3)] \\ & \quad + 4\bar{\alpha}_1\bar{\alpha}_2[V(\alpha_3)C(\alpha_1, \alpha_2) + 2C(\alpha_1, \alpha_3)C(\alpha_2, \alpha_3)] \\ & \quad + \bar{\alpha}_3^2[V(\alpha_1)V(\alpha_2) + 2C^2(\alpha_1, \alpha_2)] \\ & \quad + \bar{\alpha}_2^2[V(\alpha_1)V(\alpha_3) + 2C^2(\alpha_1, \alpha_3)] \\ & \quad + \bar{\alpha}_1^2[V(\alpha_2)V(\alpha_3) + 2C^2(\alpha_2, \alpha_3)] \\ & \quad - \bar{\alpha}_1^2C^2(\alpha_2, \alpha_3) - \bar{\alpha}_2^2C^2(\alpha_1, \alpha_3) - \bar{\alpha}_3^2C^2(\alpha_1, \alpha_2) \\ & \quad - 2\bar{\alpha}_1\bar{\alpha}_2C(\alpha_1, \alpha_3)C(\alpha_2, \alpha_3) \\ & \quad - 2\bar{\alpha}_1\bar{\alpha}_3C(\alpha_1, \alpha_2)C(\alpha_2, \alpha_3) \\ & \quad - 2\bar{\alpha}_2\bar{\alpha}_3C(\alpha_1, \alpha_2)C(\alpha_1, \alpha_3)] \\ & + 2\delta_{32}\delta_{21}[V(\alpha_3)C(\alpha_1, \alpha_2) + 2C(\alpha_1, \alpha_3)C(\alpha_2, \alpha_3)] \\ & + 2\delta_{32}^2\delta_{21}[2\bar{\alpha}_2[V(\alpha_3)C(\alpha_1, \alpha_2) + 2C(\alpha_1, \alpha_3)C(\alpha_2, \alpha_3)] \\ & \quad + 2\bar{\alpha}_3[V(\alpha_2)C(\alpha_1, \alpha_3) + 2C(\alpha_1, \alpha_2)C(\alpha_2, \alpha_3)] \\ & \quad + \bar{\alpha}_1[V(\alpha_2)V(\alpha_3) + 2C^2(\alpha_2, \alpha_3)] \\ & \quad - \bar{\alpha}_1C^2(\alpha_2, \alpha_3) - \bar{\alpha}_2C(\alpha_1, \alpha_3)C(\alpha_2, \alpha_3) \\ & \quad - \bar{\alpha}_3C(\alpha_1, \alpha_2)C(\alpha_2, \alpha_3)], \end{aligned} \quad \text{(A9)}$$

$$I_{13} = \delta_{32}\delta_{21}[V(\alpha_1)C(\alpha_2, \alpha_3) + 2C(\alpha_1, \alpha_2)C(\alpha_1, \alpha_3)], \quad \text{(A10)}$$

and

$$\begin{aligned} I_{23} = {} & \delta_{32}\delta_{21}[V(\alpha_2)C(\alpha_1, \alpha_3) + 2C(\alpha_1, \alpha_2)C(\alpha_2, \alpha_3)] \\ & + \delta_{32}\delta_{21}[C(\alpha_1, \alpha_2)C(\alpha_2, \alpha_3) + V(\alpha_2)C(\alpha_1, \alpha_3)] \\ & + \delta_{32}\delta_{21}^2[2\bar{\alpha}_2[V(\alpha_1)C(\alpha_2, \alpha_3) + 2C(\alpha_1, \alpha_2)C(\alpha_1, \alpha_3)] \\ & \quad + 2\bar{\alpha}_1[V(\alpha_2)C(\alpha_1, \alpha_3) + 2C(\alpha_1, \alpha_2)C(\alpha_2, \alpha_3)] \\ & \quad + \bar{\alpha}_3[V(\alpha_1)V(\alpha_2) + 2C^2(\alpha_1, \alpha_2)] \\ & \quad - \bar{\alpha}_3C^2(\alpha_1, \alpha_2) - \bar{\alpha}_2C(\alpha_1, \alpha_3)C(\alpha_1, \alpha_2) \\ & \quad - \bar{\alpha}_1C(\alpha_1, \alpha_2)C(\alpha_2, \alpha_3)]. \end{aligned} \quad \text{(A11)}$$

The three-way (additive × additive × additive) epistatic genetic variances for $M_3$ is defined as:

$$\begin{aligned} J_{33} = {} & \delta_{32}^2\delta_{21}^2[+2V(\alpha_2)C^2(\alpha_1, \alpha_3) + 8C(\alpha_1, \alpha_2)C(\alpha_1, \alpha_3)C(\alpha_2, \alpha_3) \\ & + 2V(\alpha_1)C^2(\alpha_2, \alpha_3) + 2V(\alpha_3)C^2(\alpha_1, \alpha_2) \\ & + V(\alpha_1)V(\alpha_2)V(\alpha_3)]. \end{aligned} \quad \text{(A12)}$$

All other three-way interaction variances have a value of zero by definition.

The environmental variances and covariances are defined as:

$$\begin{aligned} E_{33} = {} & V(\epsilon_3) + \delta_{32}^2[\bar{\alpha}_3^2 V(\epsilon_2)] + \delta_{32}^2\delta_{21}^2[\bar{\alpha}_2^2\bar{\alpha}_3^2 V(\epsilon_1)] + 2\delta_{32}\bar{\alpha}_3 C(\epsilon_2, \epsilon_3) \\ & + 2\delta_{32}\delta_{21}[\bar{\alpha}_2\bar{\alpha}_3 C(\epsilon_1, \epsilon_3)] + 2\delta_{32}^2\delta_{21}[\bar{\alpha}_3^2\bar{\alpha}_2 C(\epsilon_1, \epsilon_2)], \end{aligned} \quad \text{(A13)}$$

$$E_{13} = C(\epsilon_1, \epsilon_3) + \delta_{32}\bar{\alpha}_3 C(\epsilon_1, \epsilon_2) + \delta_{32}\delta_{21}\bar{\alpha}_2\bar{\alpha}_3 V(\epsilon_1), \quad \text{(A14)}$$

and

$$\begin{aligned} E_{23} = {} & C(\epsilon_2, \epsilon_3) + \delta_{32}\bar{\alpha}_3 V(\epsilon_2) + \delta_{32}\delta_{21}\bar{\alpha}_2\bar{\alpha}_3 C(\epsilon_1, \epsilon_2) \\ & + \delta_{21}\bar{\alpha}_2 C(\epsilon_1, \epsilon_3) + \delta_{32}\delta_{21}\bar{\alpha}_2\bar{\alpha}_3 C(\epsilon_1, \epsilon_2) \\ & + \delta_{32}\delta_{21}^2\bar{\alpha}_2^2\bar{\alpha}_3 V(\epsilon_1). \end{aligned} \quad \text{(A15)}$$

The G × E variances and covariances are defined as:

$$\begin{aligned} V(G \times E)_{33} = {} & \delta_{32}^2 V(\epsilon_2)V(\alpha_3) \\ & + \delta_{32}^2\delta_{21}^2[4\bar{\alpha}_2\bar{\alpha}_3 V(\epsilon_1)C(\alpha_2, \alpha_3) + \bar{\alpha}_3^2 V(\epsilon_1)V(\alpha_2) \\ & \quad + \bar{\alpha}_2^2 V(\epsilon_1)V(\alpha_3) + 2V(\epsilon_1)C^2(\alpha_2, \alpha_3) \\ & \quad + V(\epsilon_1)V(\alpha_2)V(\alpha_3)] \\ & + 2\delta_{32}\delta_{21}C(\epsilon_1, \epsilon_3)C(\alpha_2, \alpha_3) \\ & + 2\delta_{32}^2\delta_{21}[\bar{\alpha}_2 V(\alpha_3)C(\epsilon_1, \epsilon_2) \\ & \quad + 2\bar{\alpha}_3 C(\alpha_2, \alpha_3)C(\epsilon_1, \epsilon_2)], \end{aligned} \quad \text{(A16)}$$

$$V(G \times E)_{13} = \delta_{32}\delta_{21}V(\epsilon_1)C(\alpha_2, \alpha_3), \quad \text{and} \quad \text{(A17)}$$

$$\begin{aligned} V(G \times E)_{23} = {} & 2\delta_{32}\delta_{21}C(\alpha_2, \alpha_3)[C(\epsilon_1, \epsilon_2) + C(\epsilon_1, \epsilon_2)] \\ & + \delta_{32}\delta_{21}^2[2\bar{\alpha}_2 V(\epsilon_1)C(\alpha_2, \alpha_3) + \bar{\alpha}_3 V(\epsilon_1)V(\alpha_2)]. \end{aligned} \quad \text{(A18)}$$

The addition of the third module changes the behavior of the quantitative genetic variances. First, if we examine the additive genetic variance and covariances for the trait at the third time step, we see that the general structure of the variances is similar to that presented for trait 2. However, the variance and covariances are now contingent upon the location of the population in three-dimensional genetic space (i.e., depends on the means of all three intrinsic components) and also depends on the covariance between all intrinsic effects.

Whereas the additive genetic variance and covariances have a similar structure to those associated with $z_2$, the epistatic variance and covariances are very different. Epistatic variance now depends on the location of the population in three-dimensional genetic space. Because of this, the epistatic variance evolves as the population moves on the phenotype landscape. Thus, selection or drift can alter the relative contribution of additive and epistatic variance to phenotypic variation and modify the evolutionary potential of a population. Covariation between intrinsic genetic effects plays a particularly important role in determining the magnitude of the epistatic variance. Changes in the underlying covariance between intrinsic effects can affect the ratio of additive to nonadditive components. The addition of a third interacting unit also allows for nonadditive genetic covariances (eqs. A10 and A11). Because trait 3 shows epistatic interactions arising from nonadditive effects of $\alpha_3$ with $\alpha_2$ and $\alpha_1$, a three-way epistatic interaction now appears (see eq. A12).

Finally, we see that the addition of a third time step makes the G $\times$ E variance contingent upon the location of the population in genetic space. This provides a mechanism for the evolution of G $\times$ E variance, which may be important in models of phenotypic plasticity and environmental canalization. Nonadditive interactions during development create a situation where the G $\times$ E variance is not constant for all combinations of genetic and environmental effects.