

## Multivariate phenotypic evolution in developmental hyperspace

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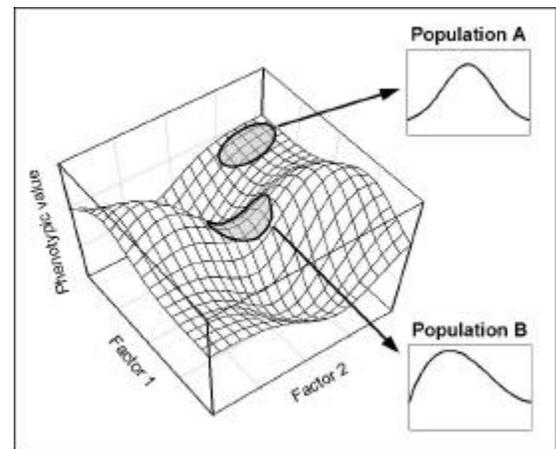
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Complex phenotypes are constructed during development by interactions among an intricate array of factors including genes, gene products, developmental units, and environmental influences. Although patterns of genetic variation and covariation play a clear role in determining patterns of phenotypic evolution (Lande 1979), it can be difficult to understand how various patterns of developmental interactions affect the evolutionary process, due to the complexity of developmental systems. However, recent advances in theory (e.g., Cowley and Atchley 1992; Atchley et al. 1994; Rice 1998, in press.; Gilchrist and Nijhout 2001; Wolf et al. 2001), and an explosion of interest in evolution and development, make it clear that a consideration of developmental interactions provides major insights into character evolution, including the evolution of phenomena such as canalization (Rice 1998), phenotypic integration (Cheverud 1996; Rice 2000), and genetic variance-covariance structure (Atchley 1984; Atchley and Hall 1991; Atchley et al. 1994; Atchley and Zhu 1997). These insights might allow one to predict (Rice in press) or reconstruct (Lande 1979; Schluter 1996) evolutionary trajectories, and to identify the proximate basis of differentiation within or among species (e.g., Stern 1998; Kopp et al. 2000; Sucena and Stern 2000).

The “phenotype landscape” (see Rice 2000) has emerged as a useful tool for understanding the way that developmental processes impact evolutionary processes, through the effects of development on phenotypic variation (Rice in press). The surface of a phenotype landscape (see Figure 1) defines the phenotype associated with a particular combination of underlying factors (e.g., single genes, multi-gene complexes, developmental modules). The topographical features of the landscape are determined by the developmental system that governs the interactions between the underlying factors. The number of underlying factors contributing to phenotypic variation defines the number of dimensions of space in which the landscape exists. In theory there is no limit to the number of underlying factors that can influence the expression of a particular trait and thus landscapes can exist in very high dimensional space (i.e., hyperspace). The terminology we use to describe the topography of a 3-dimensional landscape can be applied to hyperdimensional landscapes

but the intuitive interpretation of terms like “slope” or “curvature” become increasingly abstract as the dimensionality increases. However, this does not alter the usefulness of the approach, though it does mean that one should be cautious when interpreting the topography



**Figure 1.** An example of a phenotype landscape. The expected phenotypic value of an individual is a function of the value of two underlying factors (A and B) that interact during trait development. Populations may lie anywhere on this surface. Two populations (shaded regions) are shown, each has a different mean value for factors A and B. The phenotype distribution of each population is defined by the mean and variance of factors A and B; phenotypes in Population 1 are normally distributed but in Population 2 the phenotype distribution is skewed.

of hyperdimensional landscapes since descriptors like hilly or rugged, which have intuitive meanings in 3-dimensional space, may be misleading when applied to higher dimensional space (see Gavrillets 1997; Gavrillets and Gravner 1997).

The landscape provides a concise summary of the pattern of developmental interactions and the resulting relationship between variation in underlying factors and the phenotype. If there are multiple traits, each is expressed as a separate landscape, influenced by a common set of underlying factors. Pleiotropy is captured by the topographical relationships among such landscapes (Rice 2000; Wolf et al. 2001). The distribution of phenotypes in a population is reflected by the location of the population within the overall phenotype landscape, and movement of a population

within the phenotype landscape can be used to describe evolution, providing an intuitive understanding of various evolutionary processes (much like the adaptive landscape; Wright 1932; Wright 1977).

The location of each individual in the phenotypic hyperspace is determined by the value of its underlying factors. Because a population appears as a distribution of individuals plotted on the surface of the landscape, the population “experiences” only the region of the landscape covered by that distribution at any given time. By examining the geometry of the landscape in the local region occupied by a population, we can determine how underlying factors contribute to patterns of trait (co)variation. The slope of the landscape in this region measures the phenotypic variance associated with variation in the underlying factors; more steeply sloped regions correspond to areas of high phenotypic variance and less steep regions correspond to lower variance (Rice 2000). The additive genetic variance (and the covariance when more than one trait is considered) corresponds directly to the slope of the landscape when the underlying factors are genetic. When a landscape is curved so that the slope is not constant, the resulting variance components are weighted averages of the topography of the landscape in the region occupied by the population. Therefore, the properties of the phenotype distribution and the additive genetic (co)variances are determined by the location of the population on the landscape (Wolf et al. 2001).

Figure 1 illustrates the relationship between the landscape and properties of the phenotype distribution. Two populations that have equal distributions (bivariate normal) but different means for the underlying developmental factors are plotted on the surface. Population A, shown by the shaded circle, lies in a region of the landscape that is essentially a rising plane. Since individuals lying away from the mean are just as likely to be uphill as downhill on the landscape, the phenotypes are normally distributed in this population. However, Population B lies in a rugged region, located between two hills. As a result, its phenotype distribution is skewed towards positive values since individuals away from the mean are more likely to be uphill than downhill on the landscape. Despite the fact that the two populations have the same distribution of underlying factors (with only the means differing) and share the same developmental system, their phenotype distributions are very different. Thus, we see that, depending on where the population is on the landscape, the distribution of phenotypes need not be normal, even when the set of underlying factors is normally distributed in the population. The landscape in Figure 1 also shows that, when the topography of the landscape is rugged due to the complexity of developmental interactions, we need to know the value of both underlying factors in order to predict the phenotype of any individual or to predict the influence of either factor on phenotype expression.

To predict evolution of a population on the landscape we would need to know the relationship between the

phenotype and fitness. The fitness function (the fitness landscape when there are multiple traits) can be used to assign fitness values to each location on the phenotype landscape, creating a new landscape, which defines the relationship between the underlying factors and fitness. This composite surface (the adaptive landscape with respect to underlying factors) can then be used to predict movement of a population on the surface (see Rice in press for a general model).

In this chapter we explore the metaphor of the phenotype landscape and the insights it provides into questions about trait evolution, such as evolution of canalization and phenotypic integration. Canalization evolves when the developmental system is altered such that variation in underlying factors leads to less variation in the phenotype; canalization buffers the phenotype from genetic or environmental variation (Wagner et al. 1997; Debat and David 2001), depending on the nature of the underlying factors. Phenotypic integration evolves when traits are coinherited, i.e., when they evolve to become developmentally integrated (Wagner 1996), or when they are influenced in the same manner by underlying factors during development (Rice 2000). Our goal is to examine these issues using the phenotype landscape approach to illustrate how the conceptual model can be applied to data from empirical systems, and to suggest future analyses that will elucidate the roles of developmental interactions in phenotypic evolution. Although it is likely that the phenotype is determined by a multitude of underlying factors, meaning that development is often more complex than our models suggest, we use a simple two-factor surface to illustrate most points in the chapter for two reasons. First, landscapes with more than two factors are difficult to visualize since their surfaces exist in a hypercube, and thus do not lend themselves to the intuitive understanding that two-factor landscapes provide. Second, the basic concepts illustrated for two-factor systems apply equally well to any higher-dimensional surfaces (Rice in press).

In our presentation we focus on genetically based underlying factors such as developmental modules influenced by genetic effects. Developmental modules are the discrete subunits of organization that build phenotypes (Raff 1996; Wagner 1996). They include distinct entities like limb buds or imaginal discs, but can also include more dynamic entities such as morphogenetic fields. Modules exist within a hierarchy of organization, where each module is built from a set of interacting modules at lower levels of organization and usually contributes to modules at higher levels of organization (Wagner and Altenberg 1996; Magwene 2001). At the bottom of this hierarchy lies the genome, and at the top exists the gross phenotype, which itself can be viewed as a hierarchy of phenotypic units. We begin by examining the simplest landscapes, those in which only additive interactions among modules occur. We then explore more complex, non-additive surfaces to examine the influence of developmental interactions in evolution.

## EVOLUTION ON ADDITIVE PHENOTYPE LANDSCAPES

The assumption of additivity is the most widely used paradigm in evolutionary genetics (Falconer 1996), and so we begin by exploring how additivity between factors affects evolution with the phenotype landscape. Under the additive paradigm, the values of underlying factors (modules) simply sum to build the phenotype and consequently, the phenotype landscape is a plane with constant gradient (i.e., the landscape has no curvature). The gradient is analogous to the slope in two dimensions and is described by a vector pointing uphill on the surface in the steepest direction. The gradient determines the contribution of the underlying factors to components of phenotypic variance. Thus, on a flat landscape (zero gradient), all individuals lie at the same elevation on the landscape and consequently have the same phenotype, regardless of their values for the underlying factors. However, on a sloped landscape differences between individuals at the genetic level (different values for the factors) are translated into differences at the phenotypic level by development, leading to phenotypic variation. Planar landscapes can be flat or sloped (e.g., a rising plane). However, the gradient is, by definition, constant across the surface of a planar landscape. Therefore, given constant variation in the underlying developmental factors, the amount and components of phenotypic variation are the same at every location on a plane. Thus, the relatively simple geometry of planar landscapes also restricts the possibilities for the independent evolution of the phenotype mean and variance (Rice 2000; Wolf et al. 2001).

The most important evolutionary consequence of planar landscapes is that, regardless of where the population moves on the landscape, the relationship between underlying factors and the phenotype does not evolve (unless the shape of the landscape itself can evolve, which simply implies an additional dimension to the landscape). Because of this, changes in the mean values of factors like developmental modules cannot affect phenotypic variance on a planar landscape. As a result, if the phenotype landscape is planar, movement on that landscape involves only two possible outcomes: 1) Populations move up- or downhill on the landscape, so that the mean phenotype increases or decreases. The means of developmental factors can change as this movement occurs, but the developmental contribution to (co)variance remains constant because the gradient is constant at all locations. The rate of phenotypic evolution is determined by the gradient of the landscape; a steeply sloped landscape means that the population mean can change rapidly as the population climbs or descends the phenotype plane (Rice 2000). 2) Populations move across the landscape but remain at the same elevation. This can be visualized as a population sliding along a phenotype isocline across a landscape. Thus, the mean phenotype remains constant while the means of the developmental factors change. This can occur due to a

drift process, where changes in underlying factors balance in such a way that the changes are essentially neutral with respect to the phenotype. Again, the developmental contribution to (co)variance is constant, regardless of location on the landscape.

For genetically based factors that interact additively, neither the mapping of developmental variance to phenotypic variance, nor the genetic architecture depend on the location of the population on the phenotype landscape. As a result, genetic canalization cannot evolve since the developmental system does not allow the decoupling of underlying variation and phenotypic variation. Likewise, if multiple traits all show additive landscapes, then integration cannot evolve since the developmental relationship and the resulting covariance between traits cannot evolve. These processes require a landscape with curvature, allowing the gradient of the landscape experienced by a population to change as a population moves on the landscape.

Although strictly additive interactions between developmental factors may be relatively rare given the myriad of ways factors might interact during development (Rice 2000), additive interactions among factors may be found in the development of holometabolous insects. Most external adult structures (e.g., eyes, wings, antennae) of holometabolous insects develop from semi-autonomous modules, the imaginal discs, and interactions among these discs influence the growth rate and final size of the adult traits. From early in larval ontogeny, the sequestered fields of epidermis that comprise the discs grow slowly and somewhat independently from the rest of the insect. Later in larval ontogeny, the cell populations in the discs grow exponentially and differentiate, eventually developing into incipient adult structures. Experiments indicate that additive communication among discs probably affects disc ontogeny and ultimately, the size of the adult structures. For example, removal of the hindwing discs of the Buckeye Butterfly, *Precis coenia* increases the final size of other morphological structures developing in the same vicinity (Nijhout and Emlen 1998). If both hindwing discs are removed, the change in size of the forewings is nearly twice that resulting from the excision of just a single disc. These results suggest that discs may compete for a limited pool of resource or morphogen, and that this competition affects disc growth in a nearly additive way; the effect of disc removal is proportional to the rate at which the disc acquires or processes the factor, which is presumably correlated with disc size. Moreover, because disc removal affects the growth of only those discs in close proximity to the manipulated disc, disc-disc interactions may integrate the ontogeny of local 'neighborhoods' of developing traits. These disc-disc interactions appear to be conserved across holometabolous insects, as reflected in negative correlations among the size of morphological traits that develop in proximity to one another (Emlen 1996, 2001; Emlen and Nijhout 2000).

## EVOLUTION ON NON-ADDITIVE PHENOTYPE LANDSCAPES

The additive paradigm is predominant because, by assuming additivity, one can develop simplified models where many higher order terms vanish and the long-term dynamics of a system remain predictable. Among these models are those that form the foundations of quantitative genetic theory, which generally assumes that non-additive (i.e., epistatic) effects are absent (Roff 1997). It is important to note that the assumption of additivity is often based more on computational necessity than on biological reality. However, this assumption does not necessarily reflect biology, since many developmental systems can lead to non-additive interactions between genes, cells, tissues, or modules. Thus, incorporating nonadditive interactions into models may improve the 'biological reality' of theory. Perhaps more important than the increased biological reality is the fact that evolutionary processes that lead to evolution of variances and covariances, which are required for canalization and integration to occur, cannot occur unless nonadditive interactions are present (Rice 2000). Thus, a consideration of nonadditive developmental interactions should yield insight into how quantitative genetic parameters change during phenotypic evolution (Wolf et al. 2001).

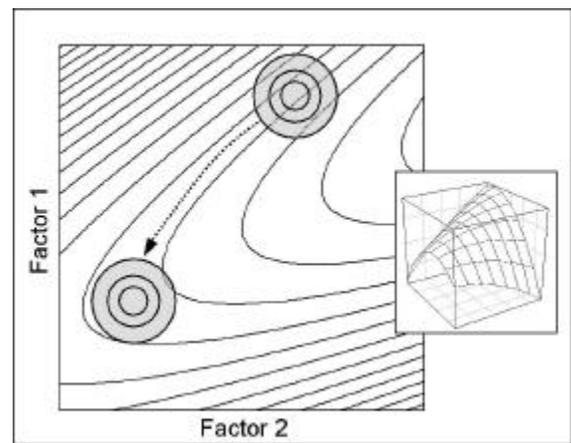
The most important implication of non-additivity of the phenotype landscape for evolution is that the phenotype distribution (characterized by variances and covariances) can evolve as the population moves on the landscape. In the following sub-sections we examine the impact of non-additive interactions between developmental factors on evolution of the phenotypic mean, the evolution of variance and canalization, and the evolution of covariance and integration. We end by considering the contribution of developmental processes to population differentiation and propose ways to apply the phenotype landscape approach to empirical questions. Throughout, we contrast evolution on additive and non-additive phenotype landscapes, paying particular attention to how the scale of population variation relative to that in the topography of the landscape affects evolutionary outcomes over the shorter and longer term.

### Evolution of the phenotypic mean

When the phenotype landscape is curved, movement on the landscape can be associated with changes in the variances and covariances that characterize the phenotype distribution (Figure 1). Evolutionary changes in (co)variance structure can occur because the curvature and average gradient are not constant across the landscape. These changes in (co)variance result from developmental interactions and can accompany evolution of the mean phenotype, as populations move uphill or downhill on a landscape. Alternatively, (co)variance can evolve independent of the mean phenotype, for example as a population moves along a contour of equal phenotype to a part of the landscape where average slope and curvature differ (Figure 2; see below). Models of

phenotypic evolution generally de-emphasize evolutionary change in genetic variance components (e.g., Lande 1979); however it is clear that if variances are to remain constant, the population must exist in a very limited parameter space where the phenotype landscape occupied by a population is invariant.

Even a very rugged landscape can appear relatively invariant if the scale of the topography is either much larger or smaller than the scale of variation in the underlying factors. The importance of scale of variation on the phenotype landscape is analogous to the problem of environmental grain in ecology (Levins 1968): populations with little variation in developmental factors relative to the scale of ruggedness in the landscape experience only the local, perhaps nearly additive (i.e., planar), portion of the landscape. In other words, due to the coarse grain of the phenotype landscape, the population does not "see" the ruggedness since the local region of the landscape is additive, and the non-linearity lies outside the range of variation present in the population (at a given point in time). As a result, populations evolve in this local region of the landscape in a fashion that is described by an additive model, even though the developmental system that produces the phenotype is not really additive. Hence, non-additivity of the phenotype landscape does not necessarily render the additive model useless. Nevertheless, as the population explores more of the topography of the



**Figure 2.** Variance can evolve independent of the mean phenotype. A population (shaded circle) starts on a steep region of the landscape, at upper right. Canalization evolves as the population, under stabilizing selection, slides along a phenotype isocline to the relatively flat area along the ridge where phenotypic variance is reduced. Note that the population starts in a location where it covers 4 isoclines and moves to a location where it essentially lies between a single pair of isoclines. In three dimensions, this landscape is a rising ridge (inset).

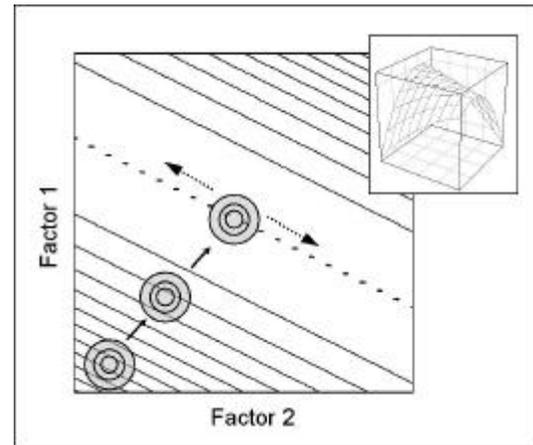
landscape over greater evolutionary time, the additive model fails. This may explain why additive models work, at least to some degree, when describing short-term dynamics but often fail over a greater evolutionary scale. The shape of the phenotype landscape determines long-term evolutionary patterns, independent of biases imposed by our limited view of the local domain occupied by a population.

Non-additivity also may be relatively unimportant when the ruggedness of the landscape occurs at a scale that is much smaller than the scale of variation. In this case, the population covers enough of the landscape to average over all of the variation introduced by the ruggedness. Hence, non-additivity adds only noise to the system in this case and therefore does not significantly alter the evolutionary dynamics (Fisher 1930). In a quantitative genetic framework, this non-additivity (i.e., the epistatic or dominance variance) is considered evolutionarily unimportant because it measures deviation from the additive model and does not contribute to heritability. Together, these considerations of scale suggest that non-additive developmental interactions remain unimportant only if populations continue to experience such equally rugged regions of the landscape through evolutionary time.

Artificial selection experiments are often used to analyze phenotypic evolution and to infer genetic variance components based on the response of a population over many generations of selection. The “realized heritabilities” (or realized genetic correlations) estimated in this way do not provide information about the shape of the landscape because realized parameters are averages, taken from the region of the phenotype landscape over which the populations traveled as the mean evolved. While stochastic variation in the response to selection is expected each generation, some of the presumably ‘stochastic’ variation observed might have important evolutionary implications - if the variation is actually due to non-linearity of the true phenotype landscape. Since the parameter estimates produced by this approach provide no information about the shape of the phenotype landscape, it is unclear how useful they are because they only describe the path already traveled by the population - they do not allow one to predict future evolutionary trajectories. For example, imagine a population evolving on a phenotype landscape that is a ridge (Figure 3), (a topography that would be due to a combination of epistasis and dominance in the developmental system; Gilchrist and Nijhout 2001; Rice 2000). If the population is selected in the direction of the ridge and starts well downhill (the first position), it can evolve for many generations in that direction (going from the first to the second position over several generations). In this case, the realized heritability would describe the average gradient of the hill climbing towards the ridge. However, that parameter is not informative as the population nears the ridge where the landscape flattens out, nor does it identify the fact that the population will reach a point beyond which the mean cannot increase.

Despite the fact that the same loci (and perhaps the same distributions of genotypic variation) contribute to phenotypic variation in different regions of the landscape, changes in variance components occur as the phenotypic mean evolves. These changes are accompanied by an apparent change in genetic and developmental architectures, despite the fact that the same developmental system maps genetic variation to

phenotypic variation in different regions of the landscape. This suggests two different views of genetic or developmental architecture: the local view, where the



**Figure 3.** Evolution on a landscape that is a rising ridge. The population (shaded circles) starts at the lower left corner, in a region that has a steep uphill gradient. Directional selection favoring larger trait values pushes the population up the hill, but as it climbs the hill the gradient of the landscape decreases. The population comes to rest on the ridge, where there is little variation produced by development. The population is now free to drift along the ridge since all locations on the ridge have the same phenotypic value. Inset: a three dimensional view of this surface.

architecture describes the pattern of genetic or developmental effects that contribute to phenotypic variation *within* a population, and the global view, where the architecture describes the entire range of variation possible. The degree to which curvature of the phenotype landscape influences the evolution of real populations remains a largely unanswered empirical question. However, the landscape view suggests that, when the ruggedness of the landscape is of the proper scale, the mapping from underlying factors to the phenotype will evolve as a population explores different regions of the landscape.

### Canalization and evolution of genetic variance

Stabilizing selection for a single optimal phenotype favors a canalized developmental system, one that reduces phenotypic variation when a population’s mean reaches the optimum. Away from the optimum, selection favors a developmental system that maps genetic variation onto phenotypic variation, allowing the population to climb the hill to a fitness peak. Thus, selection will push populations to various parts of a phenotype landscape with topography that increases population mean fitness (Rice 2000, in press), and the direction of that movement depends on the initial location of the population on the landscape. Figure 2 shows an example where a population starts in a region of a landscape that is steeply sloping (indicated by the fact that the population distribution overlaps four phenotype isoclines). If the population lies at a fitness peak, selection for canalization (i.e., low phenotypic

variance) pushes that population along a phenotype isocline to a region of the landscape that is very flat (indicated by the fact that the population now overlaps only a single isocline). Since the mean of the population moves from the first location to the second location along a phenotypic isocline, the mean phenotype remains constant while the contribution of the underlying factors to phenotypic variation is diminished (i.e., canalization is maximized).

Canalized phenotypes can evolve via factors that buffer developmental pathways against underlying genetic variation. One striking example of such a buffering system is the protein family that includes heat-shock protein 90 (Hsp90), which acts to stabilize proteins such as transcription factors that are important constituents of developmental pathways. When Hsp90 function is disrupted, by exposing developing organisms either to drugs or to extreme environments, genetic variation is revealed that produces an abnormal range of phenotypes (Rutherford and Lindquist 1998; Queitsch et al. 2002). The action of a system like Hsp90 could allow populations to move to a region of the landscape where phenotypic variation is reduced and is decoupled from underlying allelic and developmental variation. Such regulatory mechanisms that promote canalization might mask developmental divergence among populations. For example, two populations that share an ancestral, canalized phenotype might begin to accumulate subtle differences in values of developmental factors as the populations drift along phenotype isoclines in canalized regions of the landscape (like the ridge in Figure 3a). Under normal conditions, the buffering system would mask underlying variation, but extreme conditions that disrupt the buffering system could reveal underlying developmental differences between populations or closely related species. Hybridization among closely related *Drosophila* species provides strong evidence for hidden genetic variation underlying canalized phenotypes (True and Haag 2001). The shared, canalized thoracic bristle pattern exhibited by both *Drosophila melanogaster* and *D. simulans* is disrupted in the F<sub>1</sub> hybrid, and the disruption is attributed to epistatic interactions between a number of autosomal loci and a single X-linked factor (Takano-Shimizu 2000). Disruptions of the buffering system might move populations from canalized (less steep) regions of the landscape to more steeply sloped regions where underlying variation maps onto the phenotype, allowing selection to increase or decrease the mean phenotype (e.g., Queitsch et al. 2002).

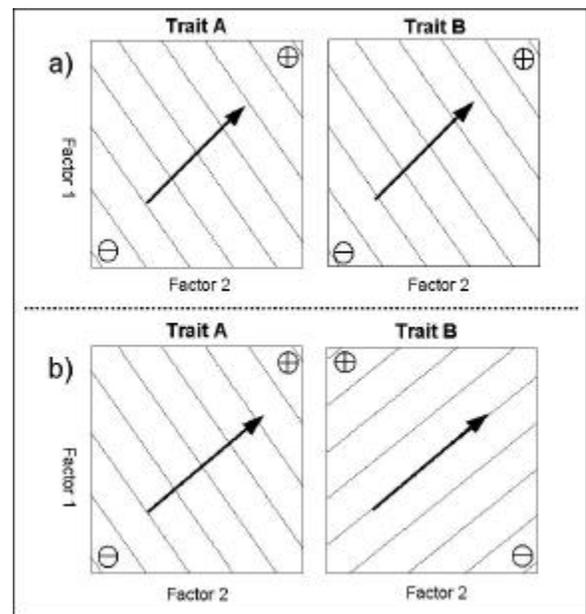
Whereas stabilizing selection can lead to canalization, other types of selection might favor increased phenotypic variance. Various forms of diversifying selection (such as disruptive selection) may push a population to a region of greater variance (i.e., a region of greater slope). Directional selection can also act to de-canalize the phenotype, by pushing a population either uphill or downhill on the phenotype landscape (depending on the direction of selection) while also pushing the population

towards a more steeply sloped region of the landscape (Rice 2000).

### Integration and evolution of genetic covariance

Integration occurs when underlying factors lead to variation in more than one trait and covariation between traits (Rice 2000). Thus, in order to understand the evolution of integration and genetic covariance we can construct landscapes for multiple traits and examine the topographical relationships between these landscapes. In the simplest case, there are two factors, each influencing two traits (in more complex multifactor space some factors might influence only one of the traits). The degree of integration between two traits is determined by the extent to which the underlying factors influence the two traits in a similar way, leading to a phenotypic correlation between the traits.

In order to illustrate the relationship between landscapes and the genetic covariance we begin with an additive example where the two factors have purely additive effects on two traits. Both phenotype landscapes are therefore planar and the same two factors define the



**Figure 4.** Additive phenotype landscapes for two traits. The same two factors influence both traits. The high point on each landscape is marked with a + sign. a) The landscapes for traits 1 and 2 are similarly sloped, leading to a positive covariance between the traits. Under directional selection for larger trait values, the two traits evolve in the same direction (arrows). b) The slope of the landscapes for traits 1 and 2 are perpendicular, leading to a zero covariance between traits (see Wolf et al. 2001). If trait 1 responds to directional selection by moving uphill, trait 2 will slide along an isocline and will not evolve (arrows).

dimensions of the landscapes. If the two planes slope in the same direction, individuals with larger values for the first trait will necessarily have larger values for the second trait and the two traits will be correlated (Figure 4a). However, if the planes are perpendicular to each other, moving uphill on one plane moves along an

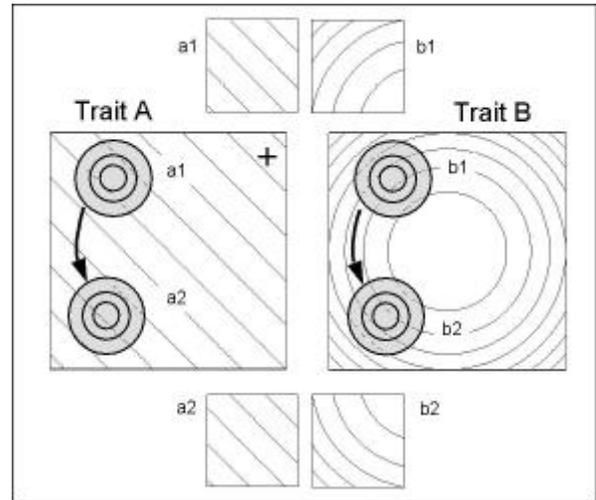
isocline of the second plane, and in this case, individuals with larger values for trait A may have any value for trait B (Figure 4b). Thus, the degree of correlation between two traits is determined by the similarity of the gradients of their phenotype landscape. This relationship holds true even if the two landscapes are not planar (Rice 2000).

For integration to evolve, only one of the traits needs to have a non-additive landscape. Therefore, we can illustrate the evolution of integration with a simple example: one trait has an additive landscape and the other has a curved landscape. If there are just two underlying factors, the correlation between a pair of traits cannot evolve unless the mean of at least one trait changes. However, it is important to note that, although we use an example where trait means change, selection for integration in higher dimensional systems may favor a system of trait development that allows trait means to remain at some optimum while genetic correlations change. Figure 5 shows two traits, where the value of trait A is an additive function of the underlying factors while the other trait shows a non-additive, peaked function. The population moves from a location of zero correlation (indicated by the a1 and b1 labels), where the gradients of the local regions of the landscapes occupied by the population are roughly perpendicular, to a region of large positive correlation (indicated by a2 and b2). This is more obvious when the local regions of the landscape are isolated and magnified, as in the insets of Figure 5. Note that the local regions of the landscape occupied by population B in both the starting and the ending location appear nearly planar in magnified view. This demonstrates that a non-additive landscape may appear nearly additive at a local scale. Also note that the value of trait B remains constant because the population moves along an isocline, while the mean of trait A evolves as the population moves downhill. This type of phenotype landscape, where the pattern of genetic covariance can evolve due to changes in pleiotropic relations, has been demonstrated by Cheverud (2001) in an analysis of QTL effects on the mouse mandible and has been termed “differential epistasis”.

The results shown in Figure 5 imply that the genetic correlation between two traits can be very labile, evolving as the populations move through phenotypic hyperspace. Although conserved genetic covariance patterns may constrain evolutionary trajectories (e.g., Schluter 1996), a number of studies demonstrate that strong directional (e.g., Leamy and Atchley 1984; Roff et al. 2002) or antagonistic selection (e.g., Sheridan and Barker 1974; Weber 1990) can disrupt covariance structure over relatively short time periods (but see Chapter XX by Pigliucci in this volume). Current theory does not allow us to predict when changes in covariance structure will occur, or whether covariance will increase, decrease, or remain constant under selection (Turelli 1988). However, viewing evolution on a phenotype landscape shows that properties of the underlying

developmental system can have important effects on genetic covariance.

On particularly well understood system, where there is considerable information available on both trait development and evolution is the expression of color patterns on butterfly wings. A number of studies have revealed the presence non-additive developmental landscapes. Experimental evolution resulting from



**Figure 5.** Phenotype landscapes for a pair of traits influenced by the same set of factors. Trait A is an additive function of these factors, and Trait B is a non-additive function of the same factors. The two large figures represent the landscapes, and the shaded circles represent a population evolving on the surface. The small surfaces (top and bottom) show an enlarged picture of each landscape at two locations occupied by the population. At the initial location (a1 and b1), the gradients of the two landscapes are perpendicular and there is zero covariance between the traits. As the population moves to the lower left (toward location a2 and b2), trait A evolves downhill on the landscape while trait 2 slides along a phenotype isocline. As the population moves, the values of the underlying factors evolve, and the covariance between the traits changes. At the ending location (a2 and b2 on the respective landscapes), there is positive covariance between traits A and B.

selection on these traits has also demonstrated considerable evolutionary lability of genetic covariance structure. Many studies focus on one kind of taxonomically widespread color pattern, sets of concentric colored rings typically called “eyespot” (reviewed by Brunetti et al. 2001). Individual eyespots appear to be separate traits influenced by a number of identifiable underlying factors. At the most basic level, these interactions are non-additive because the color exhibited in different regions of the eyespot is determined by the level of morphogen individual wing cells are exposed to, and the non-linear threshold response of the cells to the morphogen (reviewed in Brunetti et al. 2001; Beldade and Brakefield 2002). Within a wing, groups of eyespots are integrated, showing coordinated expression, with positive correlations among groups of eyespots within specific hypothesized regulatory regions (reviewed in McMillan et al. 2002). These correlations, however, are not barriers to the response to selection of at least some eyespot characters in even the short term. The high genetic

correlation among eyespots in size, for example, can be broken by disruptive selection on different size combinations among eyespots (Beldade and Brakefield 2002). The developmental basis of variation in eyespot expression has been well characterized (e.g., Nijhout 1980; Carroll et al. 1994; Weatherbee et al. 1999; Beldade and Brakefield 2002; reviewed in Brunetti et al. 2001; McMillan et al. 2002; Nijhout 1991) and so it is likely that phenotype landscapes could be constructed to predict the patterns of change found in experimental evolution experiments. The proximate basis of evolutionary change is known or strongly suggested in some cases (e.g., Beldade et al. 2002; Brakefield et al. 1996; Brakefield et al. 1998; Monteiro et al. 1994; Monteiro et al. 1997), providing a particularly powerful test of predictions.

Evolutionary lability of genetic covariance structure underlying integrated traits in natural populations has been elegantly demonstrated in the pitcher-plant mosquito *Wyeomyia smithii* (reviewed in Bradshaw and Holzapfel 2000). Populations of *W. smithii* exist along a very large south-north gradient from the Gulf of Mexico (30° N) to Saskatchewan (54° N). In the northern regions (>40° N), populations appear to have been established by a series of founder events after the last glaciation. Across the range selection favors different optimal photoperiods for diapause induction, increasing with latitude. Within populations, faster developing individuals are selected to have shorter critical photoperiods since they can complete one more generation per year than slower developing genotypes. This creates a situation where selection favors integration between critical photoperiod and development time (a positive covariance is indeed observed and is large) and shorter development times within all populations, but divergent critical photoperiods along the latitudinal gradient. From an additive viewpoint the large genetic covariance within populations would appear as a constraint on the evolution of increasing critical photoperiod as populations moved north. Although genetic correlations less than unity do not absolutely preclude the independent evolution of critical photoperiod and development time, the fact that critical photoperiod has diverged 10 standard deviations along the gradient while development has changed less than one standard deviation coupled with the relatively short evolutionary history suggests that the correlation itself does not represent a constraint (Bradshaw and Holzapfel 2000). This sort of evolutionary change matches the scenario depicted in Figure 5. A population can move to a region where the traits are not correlated, and critical photoperiod can evolve directionally while development time remains on a phenotype isocline.

Cases where the developmental basis of genetic correlations allows for rapid evolution of the correlations cannot easily be analyzed within the classic quantitative genetic framework, because this framework generally assumes constancy or proportionality of these parameters

over evolutionary time (Lande 1979). On the other hand, the understanding we achieve using the phenotype landscape approach can be more sophisticated since it is not constrained by simplifying assumptions (Rice in press). Thus, it can be used to gain insight into a number of evolutionary processes that are not accessible to classic, variance-partitioning quantitative genetics.

#### DIFFERENTIATION OF POPULATIONS

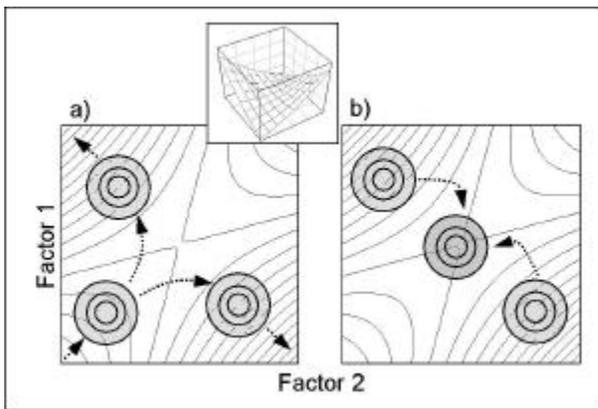
One of the most important processes in evolution is the differentiation of populations. Although population differentiation is the foundation for speciation, differentiation also plays an important role within species. Evolutionary divergence can lead to various distributions of phenotypic and genetic variation across the range of a species, to the extent that outbreeding depression can occur when differentiated populations later cross (e.g., Edmands 1999; Gharrett et al. 1999; Fenster and Galloway 2000). Such population and species differences provide snapshots of various regions of the phenotype landscape, and enhance our understanding of evolutionary processes by allowing for a larger-scale understanding of the genetic and developmental basis of phenotypic variation (Johnson 2001).

Populations can become differentiated with respect to the mean values of their phenotypes, or with respect to the values of the underlying factors. Although populations can also differentiate with respect to the shape of the phenotype landscape, this simply implies that there are additional dimensions through which populations can move, which alter the apparent shape of a landscape as viewed in reduced dimensional space. For example, if evolution at one locus alters the shape of the landscape describing the phenotype as a function of alleles present at two other loci, this third locus is simply an additional dimension. The third locus is epistatic with the two loci and the epistatic interaction appears to change the shape of the landscape in the reduced dimensional (i.e., two-locus) system.

As discussed previously, changes in underlying factors are not always accompanied by changes in the mean phenotype. Figure 6a shows an example of population differentiation, where two lineages evolve independently from a single ancestral population. The populations experience the same directional selection pressure for larger phenotypic values, but by random chance take different routes to reach the same phenotypic value at the point where we now find them. These two populations appear phenotypically identical, with the same phenotypic mean and variance. However, the genetic basis for the phenotype is completely different. Crossing these two populations would produce a genetically intermediate population that would lie in a valley of lower phenotypic value, and thus, of lower fitness (since there is directional selection for larger values) (Figure 6b). In this case, outbreeding depression results because hybridization breaks up the

developmentally integrated (i.e., coadapted) combinations of factors.

One important result of artificial selection analyses is that replicate populations exposed to uniform selection (of equivalent intensity and direction) often reach the same phenotypic endpoint via very different genetic or developmental changes. For example, replicate lines of mice selected for increased tail length (Rutledge et al. 1974) differed with respect to the underlying mechanism, but not the degree, of tail elongation: one replicate increased the number of tail vertebrae, while the other increased the size of individual vertebrae without increasing vertebral number. This response is similar to that shown in Figure 6a. In response to directional



**Figure 6.** Population differentiation in a saddle-shaped phenotype landscape. a) A population starts at the lower left corner (the dark circle) and directional selection for larger trait values pushes it up hill (illustrated by the dashed arrows). As the population reaches the saddle area it can move up hill on either side of the saddle since the landscape is symmetrical. The two alternatives are shown in the upper left and lower right regions. b) Two populations that took different routes up the saddle are differentiated with respect to underlying factors but have the same phenotypic mean since they lie at the same elevation on the landscape. Crossing these two populations (illustrated by the dashed arrows) produces a hybrid population that lies at the saddle point and, because it has a lower phenotypic value, it has low fitness. Inset: a three dimensional view of this surface.

selection, the two replicate populations can move independently to regions of the phenotype landscape characterized by identical tail lengths but different combinations of underlying factors that control the growth or condensation of vertebral elements that make up the tail. In this case, the direct response to selection (tail elongation) is the same in the two populations, but the correlated response to selection (change in the size or number of vertebrae in response to selection on tail length) differs between the populations. Additional work on murine skull characters (e.g., Atchley et al. 1982, 1990) elegantly demonstrates that selection on whole-body traits such as weight gain or fat content produce predictable direct responses to selection, but unpredictable responses of correlated skull and mandible characters. Variation in the correlated response among replicate populations can be traced to divergent developmental mechanisms among replicates, such as differences in the onset, offset, and duration of weight

gain and changes in timing of interactions between skeletal elements.

Although the developmental basis for many quantitative traits is still poorly understood, unpredictability of the correlated response is a common outcome of artificial selection experiments (Bohren et al. 1966; Gromko et al. 1991). The correlated response may bear little resemblance to the response predicted from genetic correlation estimates in the base population, and can differ markedly in both sign and magnitude between replicate populations exposed to the same intensity and direction of selection (Deng et al. 1999; Gromko et al. 1991; Palmer and Dingle 1986). Differences in the correlated response to selection alter patterns of phenotypic correlation among characters (Atchley et al. 1990) and may alter the long-term trajectories of populations by exposing new kinds of variation to selection. From the viewpoint of traditional quantitative genetics, such results highlight the limited usefulness of parameters such as the genetic correlation for making predictions about response to selection, and underline the need for models with enhanced predictive power.

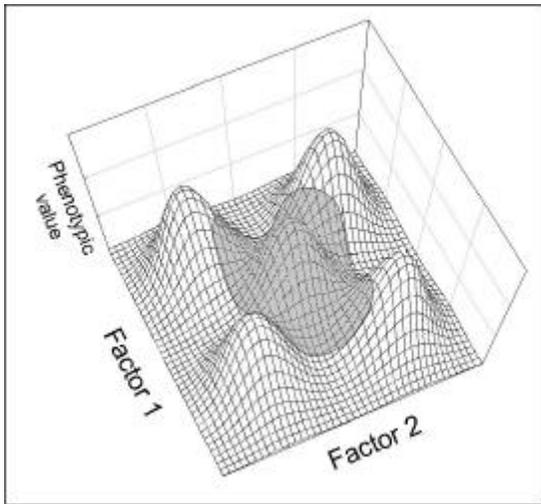
#### EMPIRICAL ANALYSIS

The phenotype landscape approach has been used primarily as a tool to develop theory—to describe the potential for developmental interactions to impact evolutionary processes through the evolution of canalization (Rice 1998, 2000) and through impacts on genetic variance and covariance that determine the evolutionary trajectory of populations (Wolf et al. 2001). Whether these advances in theory lead to critical insights or a better ability to predict evolutionary outcomes depend on the application of phenotype landscape models to empirical questions. Critically, researchers must be able to characterize landscapes in real systems (see Rice in press) if the phenotype landscape approach is to be useful. Understanding developmental landscapes requires the ability to predict the resulting phenotype from a given combination of underlying genotypes. However, because a real population may occupy only a small region of space on the landscape it may be difficult to characterize the shape of the landscape by examining only the region currently occupied by a population.

There are two basic ways to overcome this limitation, and both have been previously suggested for dealing with similar limitations in analyses of selection surfaces (Arnold et al. 2001; Phillips and Arnold 1989). The first is to maximize the distribution of individuals across the landscape, sampling in a way that avoids over sampling any one part of the distribution of underlying factors. This method contrasts with the usual approach of random sampling from a population, which biases the estimate of surface shape to reflect the space around the mean, where most of the population lies. The region around the mean is generally not the region of the landscape that most interests us for evolutionary analyses.

For example, consider a population that lies at the top of a peak on the landscape in a region where there are

many surrounding peaks (Figure 7), which may be common if multiple combinations of underlying factors can produce a large phenotypic value. Since most of the population lies near the top of the peak, the estimated surface will appear to simply have a positive curvature since the region around the mean will determine the apparent shape. However, choosing individuals from across the distribution and weighting all classes equally would provide a less biased estimate of the shape of the surface. In this hypothetical example, one might then detect the negative curvature at the edge of the range.



**Figure 7.** A rugged phenotype landscape with many peaks. A population is shown as the shaded region on the landscape. Because the population mean lies on the central peak, random sampling will underestimate the curvature of the landscape.

Although it yields a better picture of the landscape, this approach is still inadequate for some evolutionary analyses because the range of available values for the underlying factors and the phenotypes limits our view of the landscape. This limitation may be overcome using the second of these approaches, where the range of values for the underlying factors is expanded by experimental manipulation. In this experimental approach, one may either modify the values of the underlying factors to values outside of their naturally occurring ranges or modify values of some factors to produce combinations of factors that do not occur naturally. One such approach, called phenotypic engineering, has been successfully used in selection studies, giving researchers a broader picture of the fitness landscape (Sinervo and Huey 1990; Ketterson et al. 1992; Sinervo et al. 1992). There are a variety of techniques that can be brought to bear on this approach such as direct manipulations of development by removing glands or applying exogenous hormones, using mutants (see Chapter XX by Pigliucci in this volume) or transgenics, and culturing cells or organs in different physiological cocktails. Returning to Figure 7, we would expect the phenotypic engineering approach to reveal the true ruggedness of the landscape, if we were

able to create individuals in the regions beyond that currently occupied by the population.

We can gain a number of significant insights from a more complete understanding of the phenotype landscape. For example, under directional selection, our population might become trapped on a phenotype peak, or, when considering higher-dimensional spaces, in a local maximum that is not connected to other high fitness regions. When this occurs, a population may be unable to evolve to a higher peak because moving to the higher peak by directional selection would require the population to evolve through a phenotype valley associated with lower fitness. These phenotype peaks, created by non-additive interactions in the genetic and/or developmental system, may not be identified using traditional quantitative genetic techniques, but could have a major impact on character evolution. The phenotype landscape approach could provide insights by identifying these topographic features that affect evolutionary trajectories.

The engineering approach may also provide much needed insight into the evolution of canalization, if certain nonadditive topographies reveal situations where genetic (i.e., additive, dominant and epistatic) and environmental variances are labile characteristics of a population, easily evolving to new values. The engineering approach might provide important insights into multivariate evolution by revealing the mechanistic basis for genetic correlations, allowing us to understand how integration evolves. It is also likely that, by understanding the developmental basis of the genetic correlation, we would be able to understand to what degree it represents a constraint or is, again, a labile characteristic of a population, molded by functional relationships between traits to achieve patterns of integration.

Finally, the landscape itself can replace quantitative genetic parameters when modeling evolutionary dynamics. It is a particularly powerful method because it requires few or no simplifying assumptions about the shape of the landscape used to analyze trait evolution. Rice (in press) presents an eloquent model in which the geometry of the hyperdimensional landscape can be used to explore how evolution alters any moment of a phenotype distribution, from the mean and variance to more complex moments such as skewness and kurtosis. The model interfaces well with the sorts of data that are derived from developmental studies, such as changes in trait values as a function of factors such as morphogen concentrations. Developmental modules of all sorts are often useful underlying factors since their contribution to the gross phenotype is often obvious, and experimental analyses of epigenetic interactions between modules may be feasible. Rice also suggests that underlying factors such as QTL or gene expression patterns estimated from microarrays could be used to construct the landscape, providing a way to integrate theoretical studies with cutting edge developmental biology. The ability to predict evolutionary changes in all aspects of the

multivariate phenotype distribution using theory that integrates the complex developmental systems that build traits is likely to provide great insights into the evolution of complex traits, and may emerge as a unifying theory of trait evolution (see Rice in press).

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